AN INTRODUCTION TO BIOPHYSICS WITH MEDICAL ORIENTATION

Edited by : I. Tarján

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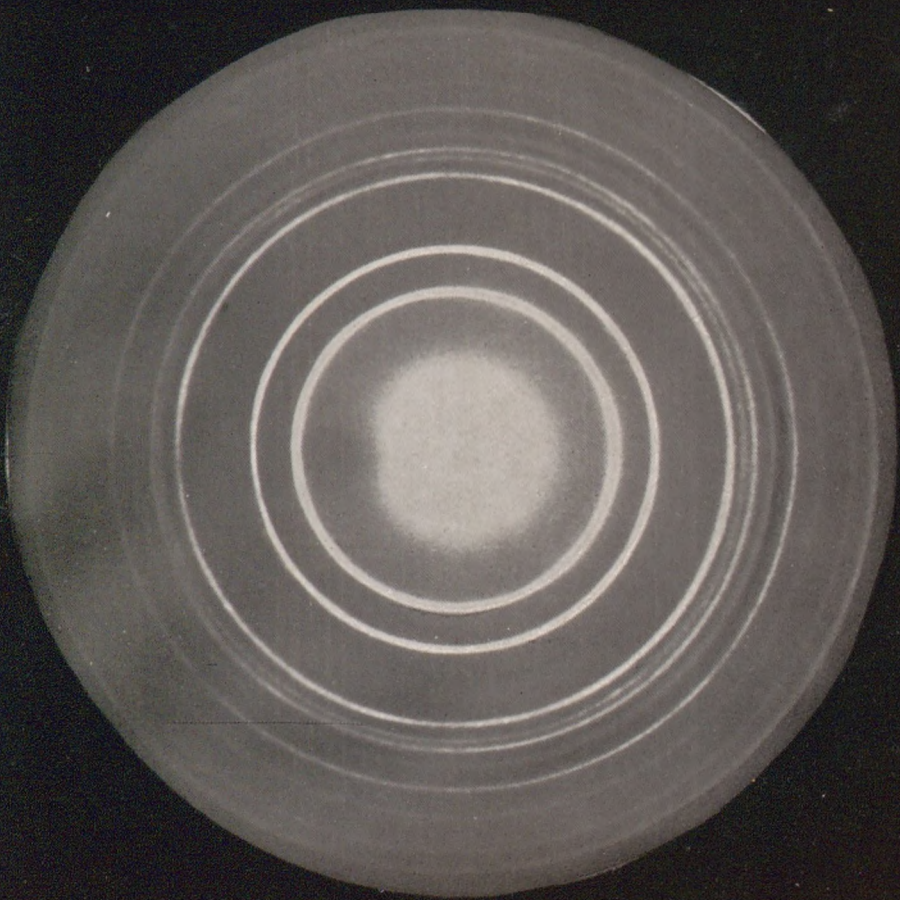
L. Berkes • S. Györgyi • G. Rontó • I. Tarján • R. Voszka



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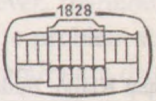

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PREFACE

Several authors agree that the field of biophysics is extremely broad and also controversial. Every book treating biophysical subjects naturally adopts the author's (authors') ideas and conceptions, which in given instances comply with the object and the circumstances.

The present book is primarily a university text book written for the purpose of medical training, but it can be also used as a manual by physicians, in some cases by biologists and also physicists and engineers who are interested in interdisciplinary problems. The book does not contain physical, chemical, biological and mathematical fundamentals, since with regard to high school studies, considering also our experiences, they can be assumed to be known to the required extent.

Our purpose has been to supply the reader with sufficient knowledge which helps the students in their theoretical medical and clinical studies, and gives at the same time some bases to continue more profound biophysical studies in several fields of this discipline.

The present revised edition contains, similarly to the previous editions, selected chapters treating the interdisciplinary fields of physics, chemical-physics, biology and medicine. In selecting the subjects of the individual chapters beside some traditional topics a few problems in the limelight of interest and pointing to the future are also dealt with.

The authors think that to discuss the structure of matter and the relation of structure and function in the more important macromolecules is highly actual. – The methods of the molecular structure analysis are dealt with in a separate chapter which reveals also the increasing importance of getting a deeper insight into the structure of matter. – A relatively extensive chapter deals with light-, X-ray- and nuclear radiations and the physical bases of their medical applications. The discussion of radioactive labelling and medical technology has been also promoted by some subjective attitude, namely that the authors used to be engaged in an active research work in this field. – At the same time, however, thermodynamics and the various transport processes present a highly up to date problem in understanding bioenergetics. The fundamental concepts of thermodynamics are discussed to a somewhat larger extent than usual in books written for physicians and biologists. The main reason of this more profound treatment is the sometimes superficial and even negligent presentation

1. THE STRUCTURE OF MATTER. MOLECULAR BACKGROUND OF STRUCTURE AND FUNCTION

The relation between the structure and function of matter is a fundamental problem of science. Research into this relation became of particular importance in biology as soon as up-to-date and sophisticated methods of structure analysis were elaborated, leading to a better understanding of the functioning of living organisms; knowledge in this field has been considerably extended by the use of highly developed optical and electron microscopes. This has resulted in profound biological studies at molecular and even atomic levels. Figure 1.1 (in the Supplement) not only summarizes the progress in the structural investigations, but also demonstrates the historical fact that new methods and a deeper insight usually call for new disciplines; these are not restricted to the emergence of a new branch of science, but are usually connected with a new approach to the reasoning, and a new scientific attitude.

The considerable development of physics in recent decades has led to much new knowledge about subatomic structures. However, since these are stable from a biological aspect, we shall discuss subatomic processes only occasionally in this chapter.

Every body, living or inanimate, is built up from elements (atoms). Molecules and complex systems of molecules, and the organization within these, are determined by atomic and molecular interactions, respectively. The various systems in part display the general properties of matter, but they also have special properties due to their composition and organization. On Earth life developed from inanimate nature over millions of years, mainly through particular combinations and interactions of light atoms. Consequently, living matter shows both *general* and *special* properties. The special properties account for the phenomena called life. In this chapter we shall deal mainly with the general properties of matter, though some of the biological aspects will also be mentioned. The biologically important methods of structure analysis will be discussed in Chapter 3.

1.1. The basic forms of matter

The knowledge relating to the basic properties of matter has increased considerably in the past few decades. Much earlier, the most important general characteristic of matter was considered to be its *corpuscular structure,* and the only type of matter

was thought to be the chemical (mechanical) substance built up from atoms. This conformed well with the later realization that even the atom has a complex structure: it is comprised of positively charged protons and neutral neutrons forming the atomic nucleus, and negatively charged electrons surrounding the nucleus in shells. According to our present knowledge the *force fields* (gravitational, electromagnetic fields, force fields within atomic nuclei, etc.) acting between microparticles, and also between the macroscopic bodies built up from these particles, are special forms of matter too. These two forms of matter, fields and corpuscular particles, are of equal importance and complement each other. Without any aim at completeness, a few empirical facts may be mentioned to demonstrate this.

(a) Under certain conditions, physical fields display corpuscular properties. As an example, the electromagnetic field, or more concretely light, may be mentioned. In some phenomena light behaves as an electromagnetic wave, but in its interactions with molecules, atoms or electrons light behaves as a corpuscle. Thus, light is both a wave and a corpuscle. Light corpuscles of given energy, mass and momentum, possessing simultaneously a well-defined frequency and wavelength, are called *light-quanta* or *photons*. The energy E, mass m and momentum I of a photon of frequency ν and wavelength $\lambda = \frac{c}{\nu}$ are related by the equations

$$E = h\nu, \; m = \frac{h\nu}{c^2}, \; I = \frac{h\nu}{c} = \frac{h}{\lambda} \qquad [1.1a\text{–}c]$$

where $h(=6.63\times10^{-34}\,\mathrm{Js})$ is Planck's constant, also called the action quantum, and $c(=3\times10^{8}\,\mathrm{ms}^{-1})$ is the velocity of light.

The interaction between nucleons (protons and neutrons) is a result of the nuclear force field, which is ineffective for other particles (e.g. electrons or photons). The quanta of the nuclear force field are the *π-mesons*, also called *pions*. While the photon exists only at the velocity of light, with zero rest mass, the mass of the pion, similarly to those of electrons or nucleons, is not zero. The rest mass of the pion is about 270 times larger than that of the electron.

(b) Under certain conditions, corpuscular particles display wave properties, which are otherwise features of physical fields. If, for instance, a radiation of high-velocity electrons passes through a thin metal film, a photographic plate placed behind this film will show interference patterns similar to those generated when X-rays are transmitted through the same object. The so-called *matter wave* belonging to a particle of momentum I has the wavelength (λ)

$$\lambda = \frac{h}{I} \qquad [1.2]$$

which corresponds to [1.1c].

(c) According to classical physics, particles and force fields may transform into each other. If, for instance, a positively charged electron (positron) collides with a negatively charged electron, the electron pair transforms into electromagnetic radiation, or more exactly into γ-photons (in most cases two γ-photons are produced in this process). The reverse process is also known: γ-radiation may produce electron pairs (pair formation).

(d) In both of its forms matter has mass and energy, and may have also momentum and angular momentum. These quantities are general characteristics of matter. In the different processes of matter, exchanges of mass, energy and momentum may occur, though the *laws of conservation* of mass, energy, momentum, angular momentum and electric charge remain valid. A generally accepted, consistent picture of matter has been developed by accepting that *matter is a corpuscular particle and a field. The particles are the quanta of the fields.*

Electrons, nuclei, photons and pions belong to the family of *elementary particles.* At present approximately 200 elementary particles are known, but the above particles play the primary roles in atomic processes.

1.2. Atoms

1.2.1. The principal characteristics of quantum theory (quantum mechanics)

The basic property that matter is both a particle and a field is frequently expressed by the term *wave-particle.* Wave-particles are not comparable with any physical body of everyday life. The wave and the particle natures of matter can be described separately by classical models, but attempts to reflect the reality of unified wave-particles by some similar descriptive model have remained unsuccessful. In spite of this, a theory has been developed which describes the behaviour of the smallest particles of matter, especially the events on an atomic scale. This theory, the *quantum theory,* also permits an understanding of numerous macroscopic properties of matter.

The earliest developed and basic part of quantum theory is *quantum mechanics,* which deals with the laws of motion of atomic particles. Quantum mechanics allowed an understanding of the structures of atoms, molecules and solids, and its importance is continually increasing as concerns the interpretation of chemical and biological processes *(quantum chemistry, quantum biology).* For this reason, mainly quantum mechanics will be referred to in the following discussions.

Another, similarly important branch of quantum theory embracing electromagnetic phenomena is *quantum electrodynamics.* The most up-to-date interpretation of the laws of motion of matter, unifying both quantum mechanics and quantum electrodynamics, is provided by the *quantum-field theory.*

Quantum mechanics sets out from the dualistic behaviour of matter, and draws its conclusions by means of mathematical reasoning, an understanding of which requires deep mathematical knowledge. A discussion of the higher mathematics involved would exceed the scope of this book. Only a few results will be summarized.

It is a consequence of the principles of quantum mechanics that the electrons in the atom cannot be in arbitrary states: their energy and angular momentum can assume only well-defined values, which means that these quantities can change only by well-defined *quantized* values. (The name quantum mechanics refers to this property.) With the aid of quantum mechanics the frequencies of emitted and absorbed radiation can be obtained in accordance with experience. Similarly, the intensity and polarization of radiation are calculable. The theory explains the rotational and vibrational properties of molecules, the atomic, electronic and molecular interactions, and the chemical bonds; together with the interpretation of the periodic system and the most important optical, electric and magnetic properties of solids, this constitutes an especially impressive result of quantum mechanics.

Beside the insufficiency of the simple visualization, another main property of quantum mechanics is, in many cases, its probability character. It is not possible to define the exact localization of an electron within the atom; only the probability distribution of its position can be given. However, this is not a deficiency of the theory, but a specific property of the microworld. It follows that the electrons do not actually revolve around their nucleus, as the planets in the solar system do, for instance. No atomic orbits exist in this sense, though the expression *atomic orbital* is used for a mathematical function, the *wave function* derived by quantum mechanical methods. Various physical quantities describing the state of the electrons can be calculated by means of this function.

Finally, quantum mechanics should be regarded as a theory which does not simply extend our knowledge, but leads to more universal natural laws than the laws of classical mechanics. It is generally accepted that classical mechanics can be regarded as a special case of quantum mechanics. This generalization can be advanced even further by stating that it is valid for the relation of quantum physics and classical physics as well.

1.2.2. Quantum numbers. The permissible energy states of the hydrogen atom

According to quantum mechanics, and in accordance with experience, several characteristics of the atomic electrons (in general the bound electrons), such as energy and angular momentum, do not vary continuously, but in a stepwise way; these properties are said to be quantized, and the electron states and their changes are described by discrete numbers. The states of the atomic electrons are determined by four numerical data, called *quantum numbers:* the *principal quantum number* (n), the *angular orbital momentum* (l), the *spin* (s) and the *magnetic quantum number* (m). Any change of state is connected with a change in at least one quantum number.

The possible states of the electrons, the atomic orbitals, show a well-defined arrangement: they form *shells* around the nucleus. The shells are at different distances from the nucleus, and are denoted (moving outwards from the nucleus) by the capital letters $K, L, M, \ldots$ *The principal quantum numbers of electrons in the same shell are identical.* The principal quantum numbers of the electrons in the $K, L, M, \ldots$ shells are successively the integers 1, 2, 3, ...

The angular orbital momentum quantum number associated with a given principal quantum number specifies the possible values of the *angular momentum* of the electron. The name refers to the outdated picture of electrons rotating around the nucleus on a circular or elliptical orbit, as had been conceived by Bohr and Sommerfeld. According to this older theory the angular momentum is equal to the product of the momentum of the rotating electron and its distance from the point-like nucleus. Quantum mechanics has proved that this picture is incorrect, though it is still considered that the electron within the atom does possess an angular momentum; however, this does not originate from rotation. For a given n the possible values of the orbital angular momentum quantum number are

$$l = 0, 1, 2, 3, \ldots, (n-1) \qquad [1.3]$$

Accordingly, for any given principal quantum number the $s, p, d, f, \ldots$ states exist. For instance, electrons characterized by the quantum number $n=2$ and $l=0$ are $2s$ electrons, and the $3p$ electrons have the quantum numbers $n=3$ and $l=1$. The magnitude of the orbital momentum for orbital quantum number l is given by

$$\hbar \sqrt{l(l+1)}$$

where $\hbar = \dfrac{h}{2\pi}$; h is the Planck constant.

For an understanding of the empirical facts it has to be assumed that, besides the orbital momentum, the electron has an additional angular momentum, due to its spinning around its symmetry axis. This momentum is the *spin*, and the electron is thought of as a small gyroscope. Though this model is incorrect, the existence of spin has been proved. (Incidentally, not only electrons, but also other elementary particles, such as photons and nucleons, possess spin.) The quantum number characterizing the electron spin is

$$s = \frac{1}{2} \qquad [1.4]$$

and the magnitude of the spin is

$$\hbar \sqrt{s(s+1)}$$

The angular momentum represents a vector with a corresponding direction, which must be considered whenever a preferred orientation in space exists, induced for instance by an external magnetic or electric field.

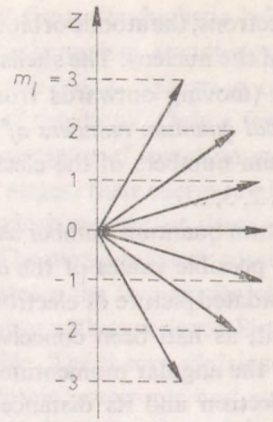

Fig. 1.2. Possible orientations of the orbital angular momentum with respect to a preferred direction (Z) for $l=3$

The only directions of the angular momentum of the atomic electrons that are permissible are those where the projection of the orbital momentum on the preferred direction is $m_l\hbar$, and the projection of the spin $m_s\hbar$ (Fig. 1.2). The quantities m_l and m_s are the *orientation or magnetic quantum numbers*. m_l may assume every integer value from $-l$ to $+l$, including zero:

$$m_l = -l, -(l-1), \ldots, -1, 0, +1, \ldots, +(l-1), +l \qquad [1.5]$$

The total number of possible values is consequently $2l+1$. The quantum number m_s has only two values

$$m_s = -\frac{1}{2} \text{ or } +\frac{1}{2} \qquad [1.6]$$

The positive sign indicates the projection in the preferred direction, and the negative sign that in the opposite direction.

The electrons have a *magnetic moment* connected with their angular momentum. The atomic magnetic moment may be conceived as the magnetic moment of revolving or spinning electric charges. However, the direction of the magnetic moment in a magnetic field cannot be arbitrary; the possible directions are determined by directional quantization.

The momentum due to the orbital momentum of an electron moving around the nucleus and to the spin can be added vectorially. The resultant total angular momentum is described by the *internal quantum number* (j), which can easily be obtained from [1.3] and [1.6]:

$$j = l \pm \frac{1}{2} \qquad [1.7]$$

In [1.7] the positive and negative signs (in agreement with experience) refer simply to the fact that the spin is only bidirectional with respect to the orbital angular momentum. Consequently, the spin either increases or decreases the orbital angular momentum quantum number by 1/2. Moreover, [1.7] states that the projection of the orbital angular momentum on any direction (in the present case on the direction of the resultant angular momentum) is an integral multiple of $\hbar$, and the projection of the spin may have only the value $\frac{1}{2}\hbar$.

Similarly as in the previous expressions, the magnitude of the resultant angular momentum is given by the quantity

$$\hbar \sqrt{j(j+1)}$$

By introducing the quantity j, the orientation or magnetic quantum number is generalized in the sense that its permissible values are

$$m = -j, -(j-1), \ldots, +(j-1), +j \qquad [1.8]$$

Therefore, there are altogether $2(2l+1)$ values (with the exception of $l=0$, since in this case j has only one value). The generalized magnetic quantum number is also called *the total magnetic quantum number* (m).

For clarity, the quantum numbers defining the permitted electron states and the exact notations of these states for the values $n=1, 2$ and 3 are listed in Table 1.1.

As an example, *the energy level system of the hydrogen atom,* which has only one electron, will now be discussed. Figure 1.3 depicts the simplified structure, which is obtained when the spin of the electron is not taken into consideration in the quantum mechanical calculations. Even with this simplification, the emission and absorption spectra of hydrogen obtained with a spectroscope of medium resolution can be suitably interpreted. The horizontal lines denote the permitted energy states of the electron. In this case these states depend only on the principal quantum number. The lowest energy belongs to the principal quantum number $n=1$; this is the ground state. The atom attains *excited states* of higher energy by energy uptake, e.g. by the absorption of photons or by collision with an electron or some other particle of appropriate energy. These processes are indicated in the diagram by arrows pointing upwards. Any transition from a higher to a lower energy state takes place by energy release, for instance by light emission or by some interaction with another particle. These transitions are shown by arrows pointing downwards. The energy values connected with the permitted transitions are presented on the vertical axes in eV units. With increasing n values the energy levels become more dense, and for $n=\infty$ an energy is obtained which corresponds to the removal of the electron from the influence of the nucleus; this process is called ionization. The amount of energy needed to ionize a hydrogen atom is 13.53 eV. If the energy uptake is higher, the removed electron has a kinetic energy equal to the excess energy. The energy of the free electron may have any ar-

Table 1.1

Quantum states of a one-electron system

Principal quantum number (n)		1	2				3								
Orbital angular momentum quantum number (l)		0	0	1			0	1			2				
Spin quantum number (s)		1/2													
Magnetic quantum number	(m_l)	0	0	0	−1	+1	0	0	−1	+1	−1	+1	0	−2	+2
	(m_s)	−1/2 +1/2													
Internal quantum number (j)		1/2	1/2	1/2	3/2		1/2	1/2	3/2		3/2		5/2		
Total magnetic quantum number (m)		−1/2 +1/2	−1/2 +1/2	−1/2 +1/2	−3/2 −1/2	+1/2 +3/2	−1/2 +1/2	−1/2 +1/2	−3/2 −1/2	+1/2 +3/2	−3/2 −1/2	+1/2 +3/2	−1/2 +1/2	−5/2 −3/2	+3/2 +5/2
Shell notation		K	L				M								
State notation		1s	2s	2p			3s	3p			3d				
Number of states		2 $2=2\times1^2$	2	6 $8=2\times2^2$			2	6			10 $18=2\times3^2$				

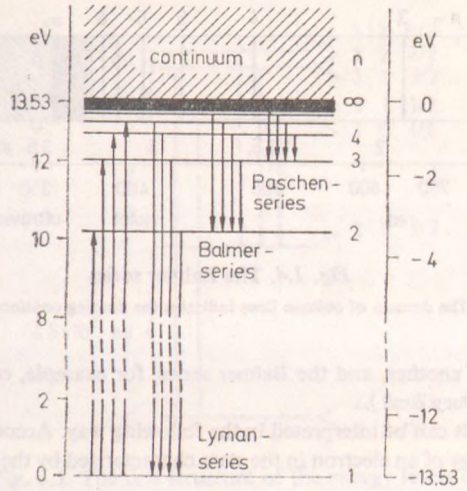

Fig. 1.3. The energy level system of the hydrogen atom
The energy of the ground state is indicated by zero on the left-hand ordinate, while the right-hand ordinate shows the energy necessary for ionization

bitrary value. This is demonstrated in the diagram by the range denoted continuum. It is generally true for any particle in a bound state that its energy can have only certain definite values, but the energy of free particles may change continuously.

The *binding energy* of an electron within the atom is defined as the energy required to remove the electron from the atom. It follows from the previous section that the binding energy is higher if the electron is closer to the nucleus, i.e. if it is located in an orbital of lower energy. Thus, the electrons in the K shell are the most strongly bound. For the hydrogen atom the binding energy of the electron in the K and L shells is 13.53 eV and 3.38 eV, respectively.

The frequencies of the spectral lines are described by simple equations. The system of spectral lines given by the same mathematical relation is called a *series*, and the equation describing this series is the *series formula*. The better-known series for the hydrogen atom are as follows:

$$\nu_{1,n} = R\left(\frac{1}{1^2} - \frac{1}{n^2}\right), \quad n = 2, 3, 4, \ldots \textit{ Lyman series} \qquad [1.9a]$$

$$\nu_{2,n} = R\left(\frac{1}{2^2} - \frac{1}{n^2}\right), \quad n = 3, 4, 5, \ldots \textit{ Balmer series} \qquad [1.9b]$$

$$\nu_{3,n} = R\left(\frac{1}{3^2} - \frac{1}{n^2}\right), \quad n = 4, 5, 6, \ldots \textit{ Paschen series} \qquad [1.9c]$$

R is the *Rydberg constant;* its value is $3.29 \times 10^{15}\ s^{-1}$. If the integers n are substituted into the above formulae, the frequencies of the series are obtained. The frequency of the first line of the best-known Balmer series (Fig. 1.4) is $4.6 \times 10^{14}\ s^{-1}$, its wavelength is 656 nm, and the emitted energy is 1.9 eV. The lines of any series are initially at a greater distance from one another; they subsequently lie

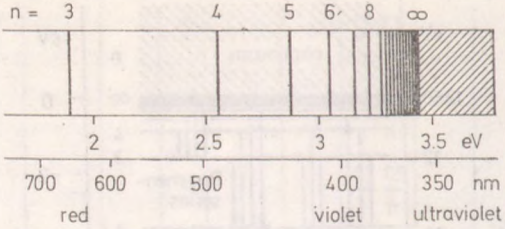

Fig. 1.4. The Balmer series
The domain of oblique lines indicates the limiting continuum

closer and closer to one another, and the Balmer series, for example, ends with $n=\infty$ at the frequency $\nu_{2,n}=R/4$ *(frequency limit)*.

The experimental facts can be interpreted in the following way. According to quantum mechanical calculations, the energy of an electron in the state characterized by the principal quantum number n is

$$E_n = -\frac{2\pi^2 e^4 m_e}{h^2}\frac{1}{n^2}, \quad n = 1, 2, 3, \ldots, \qquad [1.10]$$

where e is the elementary electric charge, and m_e is the mass of the electron. The ordinate on the right side of Fig. 1.3 corresponds to the energy values calculated from [1.10]. In order to raise the electron from the $n=1$ state into a state with $n>1$, for instance, the energy needed is

$$E_n - E_1 = \frac{2\pi^2 e^4 m_e}{h^2}\left(\frac{1}{1^2}-\frac{1}{n^2}\right), \quad n = 2, 3, 4, \ldots \qquad [1.11]$$

and the same energy is liberated when the electron returns from the $n>1$ state to the ground state $n=1$. In the case of photon absorption the energy difference $E_n - E_1$ is of the form $h\nu_{1,n}$, which leads to the equation

$$\nu_{1,n} = \frac{2\pi^2 e^4 m_e}{h^3}\left(\frac{1}{1^2}-\frac{1}{n^2}\right), \quad n = 2, 3, 4, \ldots \qquad [1.12]$$

Naturally, [1.12] also gives the frequency of the emitted light. The similarity between the empirically obtained [1.9*a*] and [1.12] calculated by means of quantum mechanics, is obvious, and the insertion of the values of the constants yields the experimentally measured R value with satisfactory accuracy. Though [1.11] and [1.12] relate to the Lyman series, the spectral lines of the other series can be interpreted in a similar way.

If the spin too is introduced into the calculations, the energy no longer depends only upon the principal quantum number, but also upon the internal quantum number. Since j may assume various values for a given value of n, the single energy level associated with n splits into several sublevels. In this way *fine structure* is obtained. Figure 1.5 illustrates the splitting of the energy levels (also called terms) of the principal quantum numbers $n=2$ and $n=3$, respectively. (Some of the lines denoting the energy levels are actually split into two, but are drawn as single lines because the levels are too close to each other.)

The magnetic quantum number becomes important in the energy terms of the

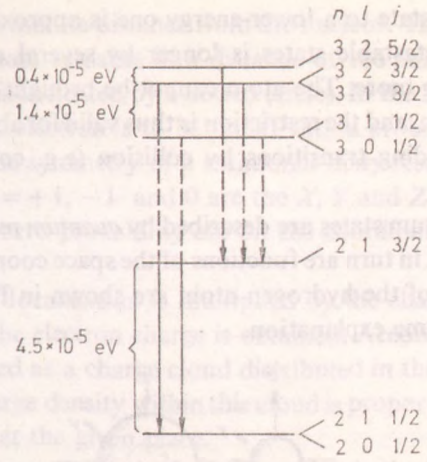

Fig. 1.5. The fine structure of the energy levels of the hydrogen atom for $n=2$ and $n=3$

electrons only if an electron localized in the electric field of a nucleus is perturbed by some other force field. This occurs when the atom is placed in a magnetic or electric field (Zeeman effect, Stark effect). Under the influence of external force fields the energy levels are split further. However, even the atomic nucleus itself may perturb the electron state, since not only the electron, but the nucleus too may have a magnetic moment, for the nucleus may also possess mechanical angular momentum. The effect of the magnetic field of the nucleus is that *hyperfine structure* is obtained. The expressions fine structure and hyperfine structure, the latter displaying even finer details, stem from spectroscopic studies, the results of which have been interpreted with high precision by the quantum mechanical method, as mentioned above.

It is found in practice that the emission and absorption spectra consist of fewer lines than expected from the calculated energy levels. This fact too can be accounted for by quantum mechanics. In an interaction with photons any change in the electron states is restricted by *selection rules*. No restriction exists for the principal quantum number (Fig. 1.3), but the orbital momentum quantum number can change only by one, and the total internal and magnetic quantum numbers must either be unchanged, or change by one.

Consequently

$$\Delta n = \text{any arbitrary integer}$$

$$\Delta l = \pm 1; \quad \Delta j = 0 \pm 1; \quad \Delta m = 0 \quad \text{or} \quad \pm 1 \qquad [1.13]$$

The arrows in Fig. 1.5 indicate the permitted transitions of light emission. Those excited states from which transition to a lower energy level is forbidden by any of the selection rules are *metastable states*. The restrictions must be interpreted statistically, which means that the probability of the transition is small. The time for spontaneous decay

from a normal excited state to a lower-energy one is approximately 10^{-8} s, whereas the life-time of the metastable states is longer by several orders of magnitude, it may be seconds or even more. The atom cannot be brought into metastable excited states by light absorption, and the restriction is thus valid for absorption too. However, there is no rule forbidding transitions by collision (e.g. collision with accelerated electrons).

The individual quantum states are described by *quantum mechanical wave functions (atomic orbitals)*, which in turn are functions of the space coordinates. Some relatively simple atomic orbitals of the hydrogen atom are shown in Fig. 1.6. The method of presentation requires some explanation.

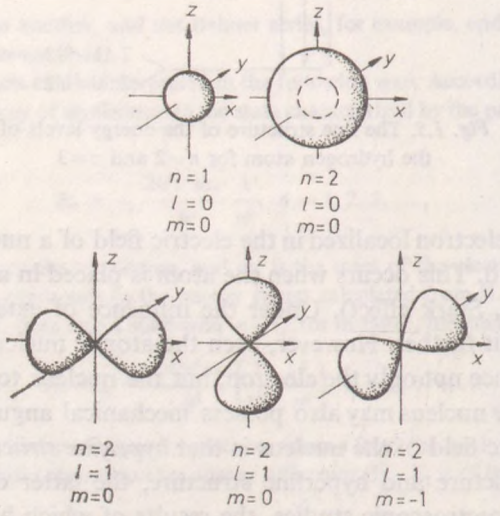

Fig. 1.6. Some atomic orbitals of the hydrogen atom

With the quantum mechanical wave functions the probability of localizing an electron at some position in the space around the atomic nucleus can be calculated. For this purpose the absolute value of the quantum mechanical wave function must be squared. The function obtained in this way describes the probability distribution of the localization of the electron. Instead of the atomic orbitals, the probability distributions are usually demonstrated by surfaces connecting points of identical probabilities around the nucleus. The various probabilities are represented by the different surfaces. Of course, surfaces may be selected within which an electron in a given state is localized with some fixed probability (e.g. 0.5 or 0.9). For simplicity, not only the wave function is called an atomic orbital (especially in connection with chemical or biological applications), but also the surface within which an electron in a given quantum state may be found with a probability of 0.9. These types of surfaces for the hydrogen atom are presented in Fig. 1.6. The *s* orbitals have spherical symmetry, the radius of the sphere containing the 1*s* electron with a probability 0.9 is 140 pm. The probability of finding the electron outside this sphere is only 0.1, but in principle

becomes zero only at an infinite distance from the nucleus. The probability of finding the electron in the $2s$ state vanishes at a distance of 106 pm from the nucleus (this spherical nodal surface is indicated by a dotted circle). In the $2s$ state the radius of the sphere within which the electron is to be found with a probability of 0.9 is 320 pm. The $2p$ orbitals show the symmetry of a rotational body resembling a figure 8. The axes of rotation for $m_l = +1, -1$ and 0 are the X, Y and Z axes of the coordinate system. The regions of zero probability are on the coordinate planes normal to the actual axis of rotation.

If the probability of localization is multiplied by the charge of the electron, the spatial distribution of the electron charge is obtained. Accordingly, the charge of an electron may be regarded as a charge cloud distributed in the space around the nucleus, and the actual charge density within this cloud is proportional to the probability of finding the electron at the given place.

So far, only the motion of a single electron moving in the force field of the atomic nucleus has been discussed (one-electron system). In a *many-electron system*, however, it is not sufficient to consider only the nucleus to describe the motion of an electron, since the effect of the other electrons, the electron coupling, must also be taken into account. From the structure of optical spectra it may be inferred that for most atoms the so called *LS-coupling (Russel–Saunders coupling)* is valid. This involves the calculation of the resultant of the orbital angular momentum and the spin momentum (**L** and **S**, respectively) for every electron separately; subsequently, the total angular momentum (**J**) of the atomic electron shell is derived from these quantities. The atomic properties are determined by the vectors **L**, **S** and **J**, where

$$\mathbf{J} = \mathbf{L} + \mathbf{S} \qquad [1.14]$$

The quantum numbers L, S and J associated with the resultant vector are usually denoted by capital letters. A relation similar to that for the one-electron system also holds for many-electron systems:

$$J = L + S \qquad [1.15]$$

L is always an integer, and quantum states corresponding to the quantum numbers $L = 0, 1, 2, 3, \ldots$ are denoted by the capital letters S, P, D, F, ... (Generally, the states of one-electron systems are described by small letters, whereas capital letters are used for many-electron systems. The S notation of the resultant spin quantum number should not be confused with the S notation of the $L = 0$ state.) Naturally, in the case of many-electron systems the resultant spin quantum number is not confined only to 1/2; it may be zero or a multiple of 1/2. As an example, consider the spins of two electrons which may be parallel or antiparallel. In the first case $S = 1$, and in the second $S = 0$. For three electrons the permissible values of S are 3/2 and 1/2, respectively. Since S and L may assume various values in relation to each other, J may assume various values for given L and S. If $L \geqq S$:

$$J = L+S,\ L+(S-1),\ \ldots,\ L-(S-1),\ L-S \qquad [1.16a]$$

and for $L \leqq S$:

$$J = S+L,\ S+(L-1),\ \ldots,\ S-(L-1),\ S-L \qquad [1.16b]$$

Thus, J can assume $2S+1$ and $2L+1$ values, respectively. This number, called the *multiplicity*, refers to the multiple splitting of the energy levels for given L and S values. With $S = 1/2$ and $L = 0$ the multiplicity is 1, but for every other L value it is 2. These are the doublet levels (including the

case $L=0$) observed in the hydrogen atom. With $S=0$ the levels are singlets, with $S=1$ triplets, and so on. The selection rules of the optical transitions are

$$\Delta L = 0 \quad \text{or} \quad \pm 1; \quad \Delta S = 0; \quad \Delta J = 0 \quad \text{or} \quad \pm 1 \qquad [1.17]$$

The selection rules account for physical properties connected with interactions between the atomic system and the angular momentum of the photon. The angular momentum of atoms has already been discussed. The photon too has spin: $s=1$. Only those interactions (emission and absorption) exist which are permitted by the law of conservation of angular momentum. The possible transitions are given by the selection rules. There is one essential condition which deserves consideration. Intense emission and absorption of radiation are possible only if the dipole moment of the system is changed in the transition. It can be demonstrated that the transitions permitted by the selection rules conform to this condition.

Calculation of the resultant momentums is facilitated by the fact that the electrons in the fully occupied shells (see section 1.2.3) can be neglected, since both the resultant orbital momentum and spin momentum are zero for these. This also holds for the electrons in the s and p states within a given shell, if these electrons constitute a closed system (saturated subshell). Consequently, only the electrons outside the saturated shells (subshells) need be taken into account.

1.2.3. The periodic system

In the periodic system (Table 1.2; cf. pp. 28–29) the chemical elements are arranged in a sequence of increasing electric charge of the nucleus or the electron shells. The *atomic number* of an element gives the number of elementary charges in the nucleus, or in the electron shells of the neutral atom which agrees with the number of protons in the nucleus and with that of the electrons in the shells. The atomic electron shell of an element in the periodic system is obtained by adding a further electron to the previous element in the system. In an atom in the ground state, every electron occupies the lowest possible energy state. It might be expected that in the ground state every electron of the atom would be situated in the K shell. However, though some of them actually are in this shell, their number is limited by the *Pauli exclusion principle*, which states that no two electrons in an atom can simultaneously have all four quantum numbers identical. The maximum number of electrons with the same principal quantum number n is $2n^2$ (cf. Table 1.1). It follows that the K shell contains at most 2 electrons, the L shell 8 electrons, the M shell 18 electrons, and so on. Table 1.3 lists the electron shell structures of the first 54 elements, giving the numbers of s, p, d, etc. electrons in the individual shells.

The expression periodic system of elements refers to the fact that several essential properties of the elements vary periodically with the atomic number. For instance, there is a striking similarity in chemical behaviour and in the nature of the optical spectra of the elements in a given column (multiplicity, cf. section 1.2.2), since the number and arrangement of the electrons in the outermost shell are identical. Thus,

the alkali metals have one electron, the alkaline earth metals two electrons, and the halogens seven electrons in the outermost shells of the neutral atoms. These are the *valence electrons* (also called photoelectrons), which determine the type of the optical spectrum. The electrons occupying lower electron shells are more strongly bound to the nucleus; these are the core electrons, which together with the nucleus form the atomic core. The differentiation of electrons into valence and core electrons is justified by the fact that the atomic core contains full (closed) shells, whereas the valence electrons occupy partly filled shells. As pointed out in section 1.2.2, the full shells have minimum energy states and the resultants of the angular momentum and the spin, and consequently of the magnetic moment are zero. For this reason the full shells are stable and virtually indifferent. This is also true within a given shell for electrons in the *s* and *p* states, for the saturated *s* and *p* subshells are closed systems. It clearly follows that the "activity" of an atom is predominantly due to electrons outside the saturated shells (subshells), and the resistance of the inert gases against chemical effects is understandable.

1.3. Molecules

1.3.1. Chemical bonds. Bond energies

Interactions between atoms are transmitted mainly by the valence electrons. Though several bond types exist, in every case the outermost electron shell is transformed so that the system attains a *stable state characterized by an energy minimum.* This holds for the formation of molecules and crystals too.

1. Bond types. The binding in *heteropolar or ionic compounds* (e.g. NaCl, CaO) results when one electron or more are transferred from one atom to another. The atom losing the electrons is transformed into a positive ion (cation), while the atom to which they are transferred is converted into a negative ion (anion). For instance, in the formation of the NaCl molecule one electron of the Na atom is transferred to the Cl atom. In this process energy is released, and Na^+ and Cl^- ions are formed. Cations and anions attract each other by electrostatic (Coulomb) forces.

The binding in *covalent* or *homopolar* compounds (e.g. most organic compounds) has been established by quantum mechanical methods. Without a rigorous discussion, a few special properties of these bonds may be mentioned. In covalent compounds electrons are not removed from the atoms; the outermost electrons of two atoms are shared between both of them and the atoms are united into one system, called a homopolar compound. For instance, two hydrogen atoms combine to yield a single H_2 molecule by sharing their electrons. The shared electrons are with high probability to be found between the two protons, holding them together in this way.

Table 1.2

Periodic system.

Ia	IIa	IIIb	IVb	Vb	VIb	VII b		VIIIb
1 H Hydrogen 1.0								
3 Li Lithium 6.9	4 Be Beryllium 9.0							
11 Na Sodium 23.0	12 Mg Magnesium 24.3							
19 K Potassium 39.1	20 Ca Calcium 40.1	21 Sc Scandium 45.0	22 Ti Titanium 47.9	23 V Vanadium 50.9	24 Cr Chromium 52.0	25 Mn Manganese 54.9	26 Fe Iron 55.8	27 Co Cobalt 58.9
37 Rb Rubidium 85.5	38 Sr Strontium 87.6	39 Y Yttrium 88.9	40 Zr Zirconium 91.2	41 Nb Niobium 92.9	42 Mo Molybdenum 95.9	43 Tc Technetium 98.9	44 Ru Ruthenium 101.1	45 Rh Rhodium 102.9
55 Cs Cesium 132.9	56 Ba Barium 137.3	57 La Lanthanum 138.9	72 Hf Hafnium 178.5	73 Ta Tantalum 180.9	74 W Tungsten 183.9	75 Re Rhenium 186.2	76 Os Osmium 190.2	77 Ir Iridium 192.2
Lanthanides:			58 Ce Cerium 140.1	59 Pr Praseodymium 140.9	60 Nd Neodymium 144.2	61 Pm Promethium 146.9	62 Sm Samarium 150.4	63 Eu Europium 152.0
87 Fr Francium 223	88 Ra Radium 226.0	89 Ac Actinium 227.0	104 257	105 257	106 263	107 262	108 264	109 266
Actinides:			90 Th Thorium 232.0	91 Pa Protactinium 231.0	92 U Uranium 238.0	93 Np Neptunium 237	94 Pu Plutonium 239	95 Am Americium 241

* The number beside the chemical symbol is the atomic number and the number below the name is the atomic mass. In the case of transuranium elements the mass number of a produced isotope is given instead of the atomic mass. At present there is still no international convention relating to the name of elements with atomic number above 103. Their mass number values are also uncertain.

of elements*

							VIII a
							2 He Helium 4.0
			III a	IV a	V a	VI a	VII a	
			5 B Boron 10.8	6 C Carbon 12.0	7 N Nitrogen 14.0	8 O Oxygen 16.0	9 F Fluorine 19.0	10 Ne Neon 20.2
			13 Al Aluminium 27.0	14 Si Silicon 28.1	15 P Phosphorus 31.0	16 S Sulfur 32.1	17 Cl Chlorine 35.5	18 Ar Argon 39.9
	Ib	II b						
28 Ni Nickel 58.7	29 Cu Copper 63.5	30 Zn Zinc 65.4	31 Ga Gallium 69.7	32 Ge Germanium 72.6	33 As Arsenic 74.9	34 Se Selenium 79.0	35 Br Bromine 79.9	36 Kr Krypton 83.8
46 Pd Palladium 106.4	47 Ag Silver 107.9	48 Cd Cadmium 112.4	49 In Indium 114.8	50 Sn Tin 118.7	51 Sb Antimony 121.8	52 Te Tellurium 127.6	53 I Iodine 126.9	54 Xe Xenon 131.3
78 Pt Platinum 195.1	79 Au Gold 197.0	80 Hg Mercury 200.6	81 Tl Thallium 204.4	82 Pb Lead 207.2	83 Bi Bismuth 209.0	84 Po Polonium 210	85 At Astatine 210	86 Rn Radon 222

64 Gd Gadolinium 157.3	65 Tb Terbium 158.9	66 Dy Dysprosium 162.5	67 Ho Holmium 164.9	68 Er Erbium 167.3	69 Tm Thulium 168.9	70 Yb Ytterbium 173.0	71 Lu Lutetium 175.0

96 Cm Curium 247	97 Bk Berkelium 247	98 Cf Californium 251	99 Es Einsteinium 254	100 Fm Fermium 257	101 Md Mendelevium 257	102 No Nobelium 255	103 Lr Lawrencium 257

Table 1.3

Electronic structures of the first 54 elements of the periodic system

Z		K	L		M			N			O	
		s	s	p	s	p	d	s	p	d	s	p
1	H	1										
2	He	2										
3	Li	2	1									
4	Be	2	2									
5	B	2	2	1								
6	C	2	2	2								
7	N	2	2	3								
8	O	2	2	4								
9	F	2	2	5								
10	Ne	2	2	6								
11	Na	2	2	6	1							
12	Mg	2	2	6	2							
13	Al	2	2	6	2	1						
14	Si	2	2	6	2	2						
15	P	2	2	6	2	3						
16	S	2	2	6	2	4						
17	Cl	2	2	6	2	5						
18	Ar	2	2	6	2	6						
19	K	2	2	6	2	6		1				
20	Ca	2	2	6	2	6		2				
21	Sc	2	2	6	2	6	1	2				
22	Ti	2	2	6	2	6	2	2				
23	V	2	2	6	2	6	3	2				
24	Cr	2	2	6	2	6	5	1				
25	Mn	2	2	6	2	6	5	2				
26	Fe	2	2	6	2	6	6	2				
27	Co	2	2	6	2	6	7	2				
28	Ni	2	2	6	2	6	8	2				
29	Cu	2	2	6	2	6	10	1				
30	Zn	2	2	6	2	6	10	2				
31	Ga	2	2	6	2	6	10	2	1			
32	Ge	2	2	6	2	6	10	2	2			
33	As	2	2	6	2	6	10	2	3			
34	Se	2	2	6	2	6	10	2	4			
35	Br	2	2	6	2	6	10	2	5			
36	Kr	2	2	6	2	6	10	2	6			
37	Rb	2	2	6	2	6	10	2	6		1	
38	Sr	2	2	6	2	6	10	2	6		2	
39	Y	2	2	6	2	6	10	2	6	1	2	
40	Zr	2	2	6	2	6	10	2	6	2	2	
41	Nb	2	2	6	2	6	10	2	6	4	1	
42	Mo	2	2	6	2	6	10	2	6	5	1	

Table 1.3 (continued)

Z		K	L		M			N			O	
		s	s	p	s	p	d	s	p	d	s	p
43	Tc	2	2	6	2	6	10	2	6	5	2	
44	Ru	2	2	6	2	6	10	2	6	7	1	
45	Rh	2	2	6	2	6	10	2	6	8	1	
46	Pd	2	2	6	2	6	10	2	6	10		
47	Ag	2	2	6	2	6	10	2	6	10	1	
48	Cd	2	2	6	2	6	10	2	6	10	2	
49	In	2	2	6	2	6	10	2	6	10	2	1
50	Sn	2	2	6	2	6	10	2	6	10	2	2
51	Sb	2	2	6	2	6	10	2	6	10	2	3
52	Te	2	2	6	2	6	10	2	6	10	2	4
53	I	2	2	6	2	6	10	2	6	10	2	5
54	Xe	2	2	6	2	6	10	2	6	10	2	6

Metals are held together by *metallic bonds*. The valence electrons of the metal atoms are removed and shared by all the other atoms, with the result that the lattice points of metal crystals are occupied by positive metal ions. The valence electrons move more or less freely in the lattice, binding together the positively charged ions. These "free" electrons are not associated with any single atom, but belong collectively to the atomic assembly. The metallic bond resembles the covalent bond in that the atoms are held together by shared electrons in both cases, but with metals the collectivization of the electrons is more pronounced.

Covalent bonds must be considered in somewhat more detail, since they are important in the structure of biological molecules. In some covalent compounds the centres of positive and negative charges coincide; these involve *pure* covalent bonds. However, a considerable number of covalent compounds are known in which the shared electrons are found with a higher probability in one part of the molecule than in another part. The centres of positive and negative charges in this case are separated from each other, and the molecules behave as electric dipoles. Covalent *dipole molecules* represent a transition between ionic molecules, which are always dipoles, and pure covalent molecules. The atoms in H_2, Cl_2 and O_2 molecules, for instance, are linked to each other by pure covalent bonds, whereas the molecules of H_2O and HCl are dipoles. Similarly, amino acid molecules, lipids and proteins involve dipole properties.

Two types of covalent bonds are distinguished, σ and π bonds. The first type is formed in the case of single bonds between electrons, be they in an *s*, *p*, or any other state. π bonds, on the other hand, are formed at multiple bonds, but one of the bonds is a σ bond. *s* electrons do not participate in the formation of π bonds.

The individual electron states and the characteristic properties (e.g. the localization, or the probability distribution) are described and calculated from quantum

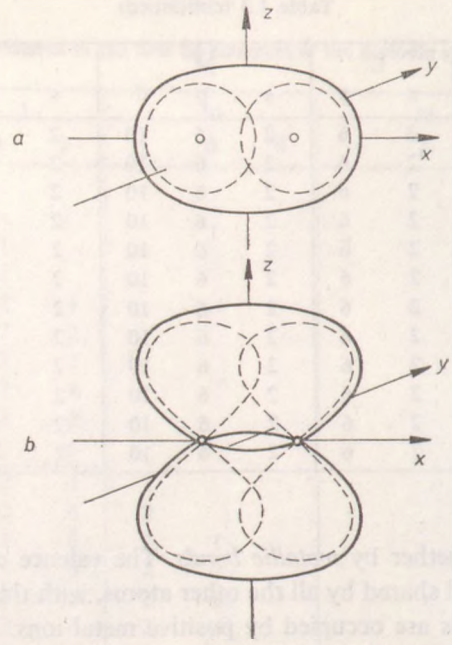

Fig. 1.7. Molecular orbitals

a: σ orbital created by coalescence of the atomic *s* orbitals, *b*: π orbital formed from the *p* orbitals. The dashed curves indicate the *s* and *p* orbitals, and the small circles denote the nuclei of the coupled atoms

mechanical functions called (analogously to atomic orbitals) *molecular orbitals.* These are conceived of in the same way as atomic orbitals.

With respect to the straight line between the nuclei of the two atoms, the bond axis, the σ orbital has the rotational symmetry depicted in Fig. 1.7*a* where X is the axis of rotation. In σ bonds the linked atoms can rotate around the bond axis with respect to each other. The π orbital has only mirror symmetry. The atomic nuclei are situated in the plane of symmetry, and the probability of finding an electron in this plane is zero. Figure 1.7*b* depicts the π orbital (with the XY symmetry plane). Atoms connected by π bonds cannot rotate around the bond axis with respect to each other. Whereas σ bonds are localized to two atoms, in multiatomic systems π electrons may be found with high probability in the vicinity of more than two atoms, i.e. they are delocalized and have *delocalized molecular orbitals.* This type of orbital can quite frequently be found in organic molecules, e.g. in the rings of aromatic compounds (see point 3 below), in proteins, nucleic acids, etc. (cf. sections 1.5.2 and 1.5.3).

2. Bond energy. One of the most important data characterizing bonds is the bond energy. This is most easily demonstrated for ionic bonds. Two ions with opposite charges are attracted by an electrostatic force, which is balanced by a repulsive force. (The repulsion will be explained later.) Both forces increase with the decrease of the

distance between the two ions, though the repulsive force increases more rapidly than the attractive one. As long as the ions are not too close to each other the attractive force is predominant; within a certain distance, however, due to its faster increase the repulsion becomes predominant. The distance at which the repulsive and attractive forces just cancel each other is called the *equilibrium internuclear distance,* and the ions oscillate around this. Instead of the attractive and repulsive forces, the energies are given in Fig. 1.8. The abscissa represents the distance (r) between two atomic nuclei, and the ordinate the interaction energy (E) between the ions. At an infinite distance E becomes zero. The potential due to the Coulomb attraction decreases hyperbolically with increasing distance (curve *a*). On the other hand, quantum mechanical calculations indicate that the repulsive energy increases exponentially with decreasing distance (curve *b*). The resulting potential (curve *c*) has a minimum at a distance r_0. r_0 corresponds to the distance where the attractive and repulsive forces are in equilibrium. In this state the ion is found in a potential valley, similarly to a ball at the bottom of a crater, and a displacement in any direction would increase its energy.

Repulsion is a tendency of two bodies to move away from each other. The repulsive potential can be deduced from Pauli's principle, since the electrons in the outermost shells of the linked atoms occupy the lowest energy state, and the shells are saturated. Any additional electrons have to be placed in a new shell, but this involves an energy uptake. If the ions came too close to each other, their electron clouds would merge. The Pauli principle would hold for the resulting system, and consequently the electrons of one of the ions should be brought to a higher energy level. The requirement of energy uptake, however, is equivalent to the development of a repulsive force, i.e. a repulsive potential.

The distance-dependence of the interaction energy in covalent bonds is described by a similar curve. The *bond energy* is defined as the energy required to remove the partner particles from their potential valley and to separate them by an infinite distance. This energy is denoted by U in the diagram. As an example $U=5.2$ eV for KCl, with $r_0=280$ pm. The same amount of energy is released when the two ions come close enough to form the KCl molecule.

The bond energy is usually given for 1 mole, and not for a single bond. In ionic, covalent and metallic bonds the bond energies are in the range of 100–400 kJ/mol, which is equivalent to 1–5 eV per bond.

For crystals, the bonds are characterized by the *lattice energy,* which is a measure of the work required to separate the lattice elements of 1 mole crystal by an infinite distance from each other.

In numerous covalent molecules the bond energy between two given types of atoms is practically independent of any other bonds which may exist in the molecule. The total bond energy of the molecule is simply the sum of all of the bond energies present. Table 1.4 lists the energies of some covalent bonds frequent in organic compounds.

When the various bond energies are additive, the bond distances between two atoms can easily be calculated as the sum of the *atomic radii* associated with the

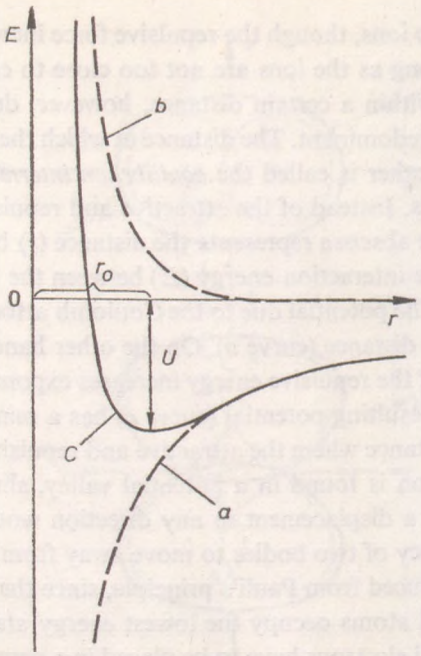

Fig. 1.8. Variation of the interaction energy (E) with the inter-ionic distance r

covalent bonds. The value of the radius depends only upon the multiplicity of the bond. Table 1.5 presents a few data; the bond length between oxygen and hydrogen atoms, for instance, is 66 pm+30 pm=96 pm.

Table 1.4

Energies of various covalent bonds

Bond	Bond energy (kJ/mol)	Bond	Bond energy (kJ/mol)
H–H	430	O–H	461
C–H	358	N–H	391
C–C	263	N–C	292
C=C	424	N–O	255
C–O	352	N–O	452

Table 1.5

Atomic radii (pm) in covalent bonds

	C	O	N	H
Single bond	77	66	70	30
Double bond	67	55	62	—

3. Conformation. Molecular conformations and crystal structures are similarly determined by the electronic structures of the associating atoms, again on the principle of *minimum energy*. We shall not go into details; only a few examples of covalent compounds will be presented.

The outermost shell of the carbon atom contains two electrons in the *s* state and two electrons in the *p* state. However, its bonds are not formed by isolated *s* and *p* electrons, but by hybridized electrons, which are equivalent from the aspect of chemical binding. The experimental fact that the four hydrogen atoms of the methane molecule, for instance, are in exactly identical positions relative to the carbon atom can be explained by the existence of equivalent bonds. The four hydrogen atoms occupy the apices of a tetrahedron, with the carbon atom at the centre (Fig. 1.9). The H–C–H bond or valence angle is 109.5°. If one of the hydrogen atoms is replaced by an OH radical, the bond angles at the carbon atom remain practically unaltered. There are only slight changes even in methyl iodide or glycine. Table 1.6

Table 1.6

Bond angles

Compound	Bond	Bond angle (degree)
Methane (CH_4)	H–C–H	109.5
Methanol (CH_3OH)	H–C–H	109.3
Methyl iodide (CH_3I)	H–C–H	111.4
Glycine (H_2N-CH_2-COOH)	C–C–N	111.8
Water (H_2O)	H–O–H	104.5
Dimethyl ether ($H_3C-O-CH_3$)	C–O–C	111
Ammonia (NH_3)	H–N–H	107.3
Trimethyl amine ($(CH_3)_3N$)	C–N–C	108

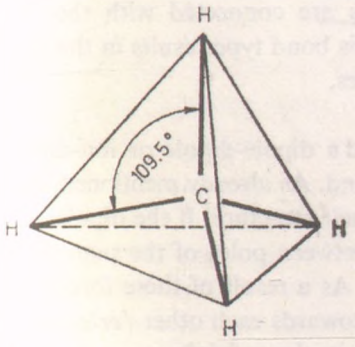

Fig. 1.9. The tetrahedral model of methane

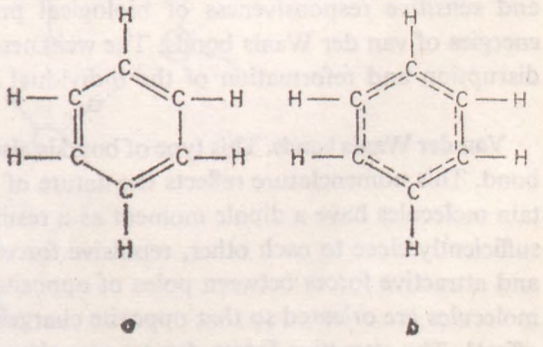

Fig. 1.10. Benzene bonds according to Kekulé (*a*) and according to experience (*b*)
The dashed lines denote delocalized π electrons

presents some bond angles, which help to illustrate the conformations of some simple molecules.

The binding in the rings or ring systems of aromatic compounds should be discussed briefly. As a simple example, benzene may be considered. According to Kekulé's formula, the benzene molecule contains six C–H, three C–C and three C=C bonds (Fig. 1.10*a*). It might be concluded that the three C=C bonds should be shorter than the three C–C bonds (cf. Table 1.6). In fact, however, all of the bonds between the carbon atoms are equivalent. This can be explained in the following way. The C–H bonds are σ bonds, and the carbon atoms are linked by six σ bonds and three π bonds. The π bonds are not localized to two adjacent carbon atoms, but are found with equal probability between any two adjacent carbon atoms. This makes the bonds between the carbon atoms equivalent. One of the usual notations is presented in Fig. 1.10*b*. This conception correctly explains the experimental results obtained for the bond energies.

1.3.2. Van der Waals bonds. Hydrogen bonds

Atoms, ions and molecules are frequently linked by van der Waals bonds. This type of bond is weaker by at least one order of magnitude (with values of 0.001–0.1 eV, or 0.08–8 kJ/mol) than ionic, covalent or metallic bonds. For this reason van der Waals bonds are usually neglected when the chemical bonds in molecules or crystals are discussed. However, in the interaction of molecules with each other or with ions and atoms, the van der Waals forces become important. The attraction between neutral gas molecules, the condensation of gases, the cohesion of the molecules in liquids, and hydrate and solvate envelopes are all due to van der Waals forces. From biological aspects these forces are of considerable importance in the formation of the secondary, tertiary, ... structures of macromolecules, and in the interactions between the structural elements of the cells. The variety, changeability and sensitive responsiveness of biological processes are connected with the low energies of van der Waals bonds. The weakness of this bond type results in the easy disruption and reformation of the individual linkages.

Van der Waals bonds. This type of bond is also called a dipole-dipole or ion-dipole bond. This nomenclature reflects the nature of the bond. As already mentioned, certain molecules have a dipole moment as a result of their structure. If the dipoles are sufficiently close to each other, repulsive forces act between poles of the same sign, and attractive forces between poles of opposite sign. As a result of these forces the molecules are oriented so that opposite charges turn towards each other *(orientation effect)*. The attractive forces due to opposite charges in close vicinity to each other lead to van der Waals binding between the molecules. However, molecules are not rigid bodies, and may become deformed in the force field of another molecule. Con-

sequently, when two molecules approach, the opposite charges within each molecule become increasingly separated compared to their original positions, which results in an increase of the original dipole moment *(induction effect)*. The strength of the bonding is thus determined by the resultant dipole moment.

At first sight it appears to be contradictory that even molecules in which the dipole moment is initially zero can be bound by dipole forces. However, it should be remembered that within an atom the positively charged nucleus and the negative electron cloud are not at rest with respect to each other. The same applies for molecules too. The centres of charges of opposite sign alternately move away from and approach each other. The overall effect is that one charged centre appears to vibrate with respect to the other centre or to rotate around it. The charges appear in positions changing with respect to each other. With molecules of zero dipole moment this means that only the average value of the moment is zero, since the centres are not coincident but are in a vibrational or rotational state. As a result of the continuous charge displacements, the molecules are actually dipoles which are associated with each other by the continually changing moment.

All this is also true for the binding between molecules and ions, molecules and atoms, or ions and atoms.

Hydrogen bonds. This type of bond is also formed by interacting dipoles. The hydrogen atom has only one electron and can form a covalent bond. In some compounds, however, the hydrogen atom can be bound to two other atoms instead of one. The additional bond is found in compounds containing fluorine, oxygen and nitrogen atoms, or FH, OH and NH radicals. The example of H_2O is presented in Fig. 1.11. The hydrogen atoms are bound to two oxygen atoms. One O–H distance is approxi-

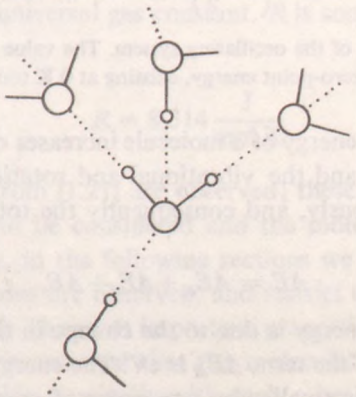

Fig. 1.11. Coupling of H_2O molecules by hydrogen bonds.
The larger circles denote oxygen atoms and the smaller ones hydrogen atoms.
The continuous lines indicate covalent bonds and the dotted ones hydrogen bonds

mately 100 pm, while the other is 180 pm. The former is a stronger (covalent), and the latter is a weaker bond: a hydrogen bond. The dipole character of the hydrogen bond is quite obvious, since the above-mentioned radicals have a relatively large dipole moment, the positive charge of the dipole being the hydrogen. This type of radical can readily bind electronegative atoms through the positive end. Hydrogen bonds play an important role in the structures of many compounds, e.g. alcohols, carboxylic acids, amines and the biologically important fats, carbohydrates, nucleic acids and proteins. The binding energy of a hydrogen bond is generally several times higher than that of a van der Waals bond.

1.3.3. The energy states of molecules

The energy of any molecule consists of the following three components (possible translational motion is not considered): the *electronic energy* (E_{el}); the energy resulting from the vibration of the molecular atomic nuclei along their connecting lines (axes), the *vibrational energy* (E_v); and the *rotational energy* (E_r), due to the rotation of the molecule itself. All these energies can have only well-defined, discrete values determined by the quantum conditions.

Let us consider a simple diatomic molecule with a structure similar to that of a dumb-bell. The molecule rotates around an axis normal to the straight line connecting the nuclei. The energy of the rotation is described by the equation

$$E_r = \frac{\hbar^2}{2K} m(m+1), \; m = 0, 1, 2, \ldots \qquad [1.18]$$

K is the moment of inertia. The atoms oscillate along their connecting axis with an energy

$$E_v = \left(n+\frac{1}{2}\right) h\nu_0, \; n = 0, 1, 2, \ldots \qquad [1.19]$$

where ν_0 is the eigenfrequency of the oscillating system. The value of E_r is never zero, even in the lowest energy state; this is the zero-point energy, existing at 0 K too.

It follows that the total energy of a molecule increases or decreases only by definite values. The electron state and the vibrational and rotational states of the molecule usually change simultaneously, and consequently the total energy gained or lost is the sum of three terms:

$$\Delta E = \Delta E_{el} + \Delta E_v + \Delta E_r \qquad [1.20]$$

The largest change in energy is due to the change in the electronic configuration. The order of magnitude of the term ΔE_{el} is eV. The energy change due to the change in the vibrational energy is smaller by one order of magnitude, and the rotational energy change is smaller by a further order of magnitude.

Basic information about the energy levels of molecules may be obtained from the *optical spectra,* though not every conceivable transition between the energy levels

appears as a spectral line. For molecules too the actual transitions are restricted by selection rules. Even so molecules present complex spectra consisting of a considerable number of lines; several vibrational states are associated with each electron state, and several rotational states with each vibrational state. If only the electron states were to change, relatively simple line spectra, similar to those for atoms, would be obtained, and the spectral lines would indicate frequencies due to changes in the state of the molecular electrons. However, the changes in the vibrational state modify every frequency, leading to multiplication of the lines. Moreover, the changes in the rotational energy yield additional modifications, resulting in further splitting of the already multiple lines. Careful study of the optical spectrum reveals further fine details, which may be accounted for by the coupling between photoelectrons (cf. section 1.2.2). The fine structure of the spectra also indicates that the moment of inertia of the molecules and their ground state frequencies are not constant, but depend upon the electronic configuration.

1.4. Gases and condensed systems. Order and disorder

1.4.1. The universal gas law and its interpretation

According to the *universal gas law (Clapeyron–Mendeleev equation)*, the product of the pressure (p) and the gas volume (V) is proportional to the product of the quantity of the gas (mole number v) and the absolute thermodynamic temperature (T):

$$pV = RvT \qquad [1.21]$$

The proportionality factor R is independent of the nature of the gas, and for this reason R is called the universal gas constant. R is sometimes also referred to as the molar gas constant.

$$R = 8.314 \frac{\mathrm{J}}{\mathrm{mol\,K}} \qquad [1.22]$$

In practice, deviations from [1.21] are observed; these are the smaller, the more point-like the molecules can be considered and the more negligible the molecular interactions. For simplicity, in the following sections we shall deal only with *ideal gases*, for which no deviations are observed, and restrict the discussion to the limiting case presented by [1.21]. The most important properties of the gaseous state can easily be understood if it is assumed that the molecules are constantly in motion *(thermal motion)*, and collide elastically with each other and with the vessel walls. Further, the molecules are assumed to move in straight lines between two collisions. The motion is totally random, in every direction and with widely varying velocities (Maxwellian velocity distribution, Fig. 1.12).

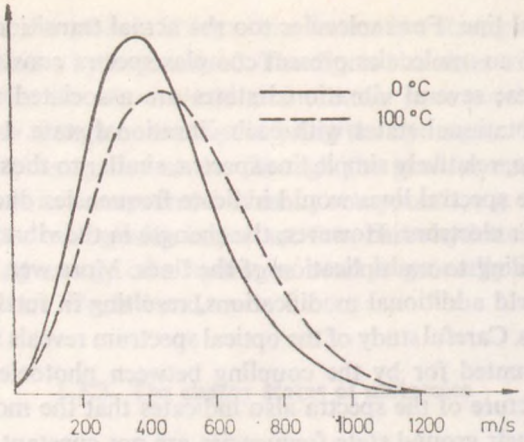

Fig. 1.12. The velocity distribution of oxygen molecules

(a) Interpretation of the gas pressure. The pressure exerted by the gas on the walls of the vessel is assumed to be a result of the elastic collision of the molecules with the walls of the vessel. If the collision of the randomly distributed molecules also occurs at random and the collision frequency is sufficiently large, the effect is manifested macroscopically by a uniform pressure distribution, produced by compressive forces acting on the walls of the vessel. The number of molecules in a comparatively small volume of gas is very high. For instance, 1 m^3 air in the normal state contains 2.69×10^{25} molecules, and even at a low pressure of approximately 10 mPa there are still 10^{18} molecules in the same volume. Calculations show that the gas pressure (p) is proportional to the concentration (n) and to the average kinetic energy of the molecules, as expressed by the mass of one molecule (μ) times the average of its square velocity ($\overline{v^2}$):

$$p = \frac{1}{3} n\mu\overline{v^2} \qquad [1.23]$$

The concentration of the molecules is defined as

$$n = \frac{N}{V} \qquad [1.24]$$

where N denotes the number of molecules in a volume V, and $\overline{v^2}$ is the arithmetic mean of the square velocities. For a given pressure and volume, n is the same for every gas *(Avogadro's law);* its value for the normal state is 2.69×10^{25} m^{-3} *(Avogadro's constant).*

(b) Interpretation of temperature. From [1.24] and [1.23]

$$pV = \frac{1}{3} N\mu\overline{v^2} \qquad [1.25]$$

If [1.25] is compared with the universal gas law [1.21]:

$$\frac{1}{3} N\mu\overline{v^2} = \nu RT \qquad [1.26]$$

The numerical value of N/ν is equal to the number of molecules per mole, thus this quotient is the same for every gas; this is the Loschmidt constant and is denoted by L:[1]

$$L = 6.02 \times 10^{23}/\text{mol} \qquad [1.27]$$

If both sides of [1.26] are divided by N:

$$\frac{1}{3} \mu\overline{v^2} = \frac{R}{L} T \qquad [1.28]$$

The quotient R/L is the *Boltzmann constant*, usually denoted by k:

$$k = \frac{R}{L} = \frac{8.31 \text{ J/mol } K}{6.02 \times 10^{23}/\text{mol}} = 1.38 \times 10^{-23} \text{J/K} \qquad [1.29]$$

Minor rearrangement of [1.28] leads to

$$\frac{1}{2} \mu\overline{v^2} = \frac{3}{2} kT \qquad [1.30]$$

which permits a more profound interpretation of temperature as well as the quantity of heat. The temperature is in a clearly-defined relation with *the mean kinetic energy of the molecules*, which *is linearly proportional to the absolute temperature*. An increase or decrease in temperature means an increase or decrease in the mean kinetic energy of the molecules. A temperature increase clearly requires additional energy, whereas a temperature decrease leads to a decrease in the mean kinetic energy of the molecules.

Volume, pressure and temperature are characteristic quantities of the *macroscopic* state of the gas, whereas the space coordinates, the velocity and the energy of the individual molecules relate to the *molecular* state. The macroscopic properties are determined by the "average behaviour" of the molecules.

[1] This nomenclature is not universally accepted; some authors interchange the *Loschmidt constant* with *Avogadro's constant*. The dimensionless numerical values of these constants are called the *Avogadro* and *Loschmidt numbers*.

If the definitions of density (ϱ) and molar mass (M) are introduced, [1.21] can be written in the following form:

$$p = \varrho \frac{RT}{M}, \quad \text{where} \quad \varrho = \frac{m}{V}, \quad M = \frac{m}{\nu} \qquad [1.31]$$

where m is the mass of a gas of volume V.

Another form sometimes used is

$$p = \varrho \frac{kT}{\mu} = nkT \qquad [1.32]$$

obtained from [1.31] using the relations

$$\varrho = n\mu, \quad M = L\mu \quad \text{and} \quad k = \frac{R}{L}$$

Thus according to [1.31] and [1.32] at a constant temperature and for a given quantity of gas, the pressure, density and molecular concentration are proportional to one another.

Some informative data on gas molecules can be summarized as follows. Any individual gas molecule undergoes collision several times within 1 s, and the number of collisions is proportional to the square of the molecular radius, the concentration of the molecules and their mean velocity. The *number of collisions per second* of a gas at atmospheric pressure and at 273 K is of the order of magnitude of 10^9.

The *mean free path length* between two consecutive collisions is inversely proportional to the square of the molecular radius and the molecular concentration. Its value at atmospheric pressure is of the order of magnitude of 10^{-8} m, but at 1 mPa it is approximately 10 m.

(c) Maxwellian velocity distribution. Figure 1.12 depicts the velocity distribution of oxygen molecules according to their velocity *(Maxwellian velocity distribution)* at temperatures of 273 K and 373 K. The abscissa represents the velocity v, while the ordinate gives the number of molecules whose velocity at some value v lies between v and $v+\Delta v$ (Δv is an arbitrarily selected small value). The maximum in the curve relates to the velocity of a relatively large number of molecules. If the velocities of the molecules were studied individually, this velocity value would be found most frequently. The most probable velocity at 273 K is around 350 m/s, and at 373 K 450 m/s. The asymmetry of the curves means that the mean velocity differs from the most probable velocity, the former being somewhat the larger. With increasing temperature the curve shifts towards higher velocities, because the number of relatively slow molecules decreases while the number of faster ones increases.

The kinetic gas theory permitted a more profound interpretation of several well-known phenomena, such as friction, heat conduction and diffusion. The experimental results are the most important evidence in support of the theory.

1.4.2. Kinetic heat theory

The molecules of the perfect gases discussed above were regarded as points, and consequently only their translational motion was considered. However, besides undergoing translation, real molecules also rotate, and moreover the atoms forming the molecules oscillate with respect to each other. For a correct interpretation of the

empirical results, not only the kinetic energy of translation, but also the kinetic energies due to rotation and vibration must be considered.

The molecules of fluids and solids (crystals)[2] too are in continuous, but irregular motion *(thermal motion)*, and it is generally true that the heating of a body is equivalent to an increase in the *mean velocity* of its molecules. In solids the thermal motion of the atomic constituents is mainly restricted to vibration and rotation around their equilibrium positions. The attractive forces keep the molecules together in liquids too, and consequently the thermal motion consists mainly of vibrations and rotations, though the migration of the molecules can no longer be neglected. With increasing temperature the structure of a liquid becomes more and more similar to that of a gas, and besides vibrations and rotations translational motion gradually comes into prominence.

Any change in phase of matter is accompanied by structural changes. The melting point is the temperature at which the concentration of the lattice defects in the crystal increases to such an extent that the lattice becomes unstable, and the degree of order decreases (cf. also sections 1.4.3 and 1.4.4). Freezing involves the opposite processes. In the case of evaporation (sublimation) molecules with relatively high kinetic energy overcome the attractive forces and break away from the liquid (solid). The average distance between the molecules in vapour is so large that their interconnection (apart from random collisions) is virtually non-existent. Condensation involves the transformations in the opposite direction.

Melting and evaporation are energy-requiring structural changes. The melting heat and the heat of evaporation supply the energy needed for the variation in the positions of the molecules that leads to the structural changes at the melting point and evaporation temperature, respectively. In freezing or condensation the opposite transformations are accompanied by heat liberation. Thus, the transformation heat is a measure of the changes in the mutual *potential energies* of the molecules.

In chemical reactions the changes in the mutual positions of the atoms (atom groups) are also accompanied by the liberation or absorption of heat (exothermic or endothermic processes). The reaction heat is a measure of the overall change in the potential energies of the combining atoms (cf. also sections 4.3.2 and 4.3.3).

1.4.3. Crystals and crystal lattice defects

Solids or crystals are discussed below, but many of the findings also apply to macromolecules (cf. sections 1.5.2–1.5.5).

As already pointed out, the order in crystals is determined by the electronic structures of the associating atoms, and is characterized by an energy minimum. In fact, perfect crystals do not exist. One reason for defective states is the thermal motion of

[2] We differentiate between "solid bodies" and "solids". The former concept refers to bodies which keep their shape and volume. (The adjective solid can frequently be substituted by hard.) The second expression, on the other hand, is equivalent to the term crystals.

the atoms, ions or molecules (i.e. the lattice elements). As a result of energy exchange between the lattice elements, some of these elements may obtain such a high energy that they overcome the attractive forces and break away from their neighbours. A small number of lattice elements of such high energy always exists at low temperatures, and this number is larger at high temperatures. For instance, evaporation or sublimation may be explained in that lattice elements with sufficiently high energy escape from the surface. This escape of lattice elements from the surface is responsible for empty lattice sites, called *vacancies,* within the crystal. When a particle has left the surface its place may be taken by a neighbouring particle from some deeper position, whose empty site in turn may be occupied by another particle from a still deeper region. In this way the vacancy migrates within the crystal (Fig. 1.13). The vacancies

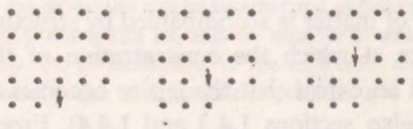

Fig. 1.13. Vacancy migration
Arrows indicate the displacement of a particle into the vacancy

created by this means are called *Schottky defects.* Vacancies are continuously created and annihilated, for instance by migrating to the surface. Crystals always contain vacancies, the number of which increases with increasing temperature. The vacancy concentration depends upon the activation energy necessary for the formation of the vacancies too. At a given temperature the vacancy concentration is lower in a material in which the activation energy is higher. At thermal equilibrium the concentration of Schottky defects (n_S) is given by

$$n_S = ne^{-\frac{\varepsilon_S}{kT}} \qquad [1.33]$$

where n is the concentration of lattice points in the material, T is the absolute temperature, ε_S is the activation energy required for the formation of a vacancy, and k is the Boltzmann constant (cf. 1.4.5). The value of ε_S lies in the range of the binding energies of the lattice elements. Near the melting points in metals, the number of vacancies is a few thousandths of the number of lattice points.

In some substances the vacancies are created not only at the surface, but also within the crystals. In this case particles leaving their lattice sites become wedged between other regular lattice sites and occupy *interstitial positions.* A defect pair consisting of a vacancy and an interstitial particle is a *Frenkel defect* (Fig. 1.14). The concentration of this type of defect pair (n_F) is given by a relation similar to [1.33].

The defect concentration can be increased above its temperature-dependent equilibrium concentration by various treatments, e.g. by deformation, by quenching

Fig. 1.14. Defect pair consisting of a vacancy and an interstitial particle

from some higher temperature, or by irradiating the crystal with some high-energy electromagnetic or corpuscular radiation. The excess of defects thus created can be conserved for some time (possibly years), though their distribution may change, the defects accumulating locally, for instance. The equilibrium concentration is restored by heating the crystal to a temperature close to the melting point, and subsequently cooling it slowly to room temperature (this cooling may last for several days).

An important group of lattice defects are the *chemical defects* created by the incorporation of foreign atoms (ions, molecules) in the crystal. These defects may be localized at regular lattice sites, or in interstitial positions, either individually or in groups (as complexes). It frequently occurs with compound crystals that some of their components are not built into the crystals in proper stoichiometric ratio.

Another type of lattice defects are *dislocations,* which are created by mechanical stresses, for instance by deformation, uneven cooling or heating, or as a result of the accumulation of vacancies or interstitial particles. Two types of dislocation exist: *edge dislocation* and *screw dislocation.* For example, if an initially regular crystal is pressed in the direction of the arrow shown in Fig. 1.15, the lattice planes are displaced with respect to one another and the result is that one plane appears to be wedged in as an extra plane not in correspondence with the adjacent planes. The lattice structure in essence becomes distorted along one line, the dislocation line, close to the edge of the extra plane. This line represents the edge dislocation. In the example in the diagram the dislocation line lies normal to the plane of the drawing, through the extension of the dashed line. Figure 1.16 shows a screw dislocation generated by a deformation acting in the vicinity of the vertical dashed line in the crystal. This line represents the screw dislocation, because a path proceeding round it (starting at point *P*) describes a spiral.

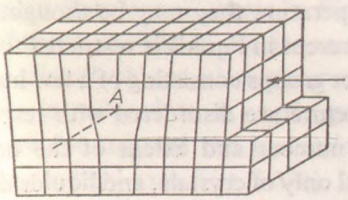

Fig. 1.15. Edge dislocation

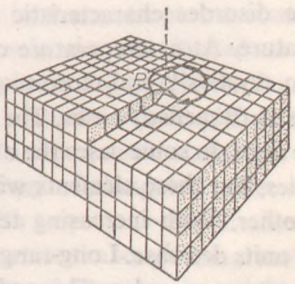

Fig. 1.16. Screw dislocation

Vacancies and interstitial particles are frequently referred to as point defects, and dislocations are also called line defects. *Surface defects* are a third type of lattice defects. The grain boundaries in polycrystalline materials are surface defects. Single crystals too may have a mosaic-like structure, consisting of crystalline blocks whose orientations differ from one another only slightly. The domains connecting adjacent blocks (block boundaries, grain boundaries) of necessity contain a large number of point and line defects, thereby allowing the evening-out of the orientational differences between neighbouring grains.

Some properties of crystals are especially sensitive to structural irregularities, e.g. diffusion, thermal conduction, plasticity, luminescence, ionic conduction, photoelectric properties, etc., i.e. all those properties connected with the migration of neutral or charged particles or energy transport.

Structural defects also exist in biological macromolecules and macromolecular systems, where the likelihood of defects is enhanced, for instance, by thread- or chain-like molecular configurations with rotational and bending possibilities. The probability of defects is especially high for the relatively weak intra- and intermolecular bonds (van der Waals bonds, hydrogen bonds). In this case too the formation of defects is a result of various factors: a temperature increase, the presence of various substances, a change in the hydrogen ion concentration, etc.

In many cases the presence of structural irregularities is unfavourable, and defect formation must be avoided or at least decreased. However, it would be a mistake to think that the irregularities result in unfavourable macroscopic or harmful functional consequences in every case. From among the examples for crystals, it may suffice to mention the basic role played by the doping additives in doped semiconductors or activated luminophores. Other well-known examples include channel formation in membranes, and the role of local denaturation in the DNA chain in connection with its biological functions (c.f. also sections 1.5.2–1.5.5).

1.4.4. Liquids and amorphous solids. Mesomorphous state

As concerns their structure, liquids comprise a transition between the solid and gaseous states. Near to the melting point liquids are still ordered to some extent, and the disorder characteristic of gases develops only gradually with increasing temperature. At a temperature close to the melting point liquids are referred to as "molten crystals", and close to the critical temperature they may be thought of as condensed (liquefied) gases. The atomic order present in liquids is restricted to small volume units. In some cases the order extends over groups consisting of a few hundred molecules, but these elements with ordered structure are disordered with respect to one another. With increasing temperature the number and extent of the ordered volume units decrease. Long-range order is typical only of crystals, and liquids display only short-range order. The ordered groups in the liquids are not stable, but are

continually breaking and reforming due to the thermal motion. The presence of the small ordered groups and their disordered orientation explains the directional independence of the physical properties of the liquids: they are said to be isotropic. Anisotropy, on the other hand, is a characteristically crystalline property.

As already mentioned, the thermal motion in liquids consists mainly of oscillations, though the equilibrium positions are less well defined than in solids. The number of vacancies in liquids is considerably larger than in solids (on melting the number is multiplied several-fold). This allows frequent displacements of particles, i.e. translational motion, which explains the fluidity of liquids.

Amorphous solids (glasses) are structurally liquids, and consequently do not have sharp, well-defined melting points, in contrast with crystals. On cooling, no qualitative change occurs in their short-range order, that characteristic of liquids simply being "frozen in". Amorphous solids are supercooled and strongly viscous liquids without fluidity.

The **mesomorphous state** is intermediate between liquids and crystals. The common liquids are isotropic in every respect, whereas crystals may exhibit anisotropy in many respects; for example the elastic constant, the dielectric constant and the optical refractive index may be direction-dependent in crystals. The *mesomorphous state* is defined as an *anisotropic liquid phase;* it is frequently referred to as *an anisotropic liquid* or *liquid crystal.*

The mesomorphous state can be observed in substances consisting of molecules with non-spherical symmetry, but of anisodimensional, e.g. rod-like or thread-like, molecules. In a consideration of the structure in the mesomorphous state not only the ordering of the molecular mass centres, but also the directional arrangement of the molecules *(translational and orientational order)* must be taken into account. The order of the intermediate phases is lower than that found in the solid phase, though higher than in real liquids.

Two types are distinguished, the classes of *thermotropic* and of *lyotropic* liquid crystals. In the former class the mesophase is induced by a temperature change. Lyotropic systems, on the other hand, are aqueous solutions of anisodimensional molecules, the phase properties depend on the concentration too. Figure 1.17 depicts the most frequently occurring arrangements found in thermotropic substances. In the *smectic* state the centres of mass of chain-like molecules are situated on planes equidistant from one another. In Fig. 1.17*a* these planes are perpendicular to the plane of the page. The molecular axes are generally normal to these planes, though oblique directions are also observed sometimes. In the *nematic* state the ordered structure is reduced to a nearly parallel arrangement of elongated molecules, no layers are formed and the molecules can move with respect to one another along their longitudinal axes (Fig. 1.17*b*). In the *cholesteric* state the longitudinal molecular axes are in parallel planes (Fig. 1.17*c* shows the planes), but axes in neighbouring

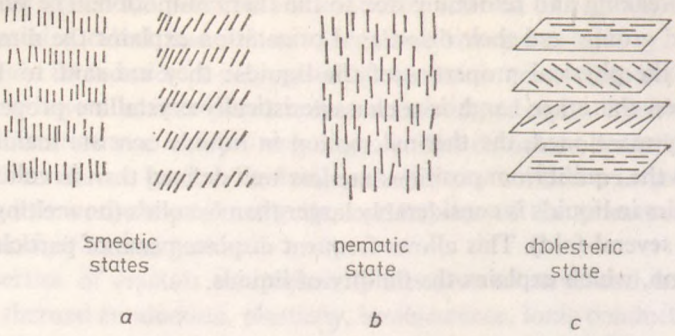

Fig. 1.17. Mesomorphous states

parallel planes are rotated with respect to one another. Consequently, the axes of the molecules in planes situated one above the other display a helical arrangement.

A sequence of decreasing order is shown in Fig. 1.18.

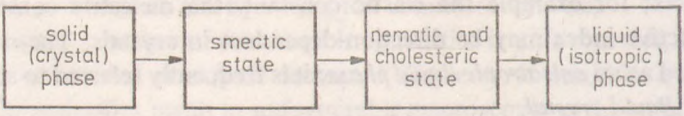

Fig. 1.18. Phase transitions in the sequence of decreasing order

Figure 1.19 depicts lyotropic systems. Soap solutions, for instance, are lyotropic systems, and nucleic acids, and many polypeptides become lyotropic when present in appropriate concentration in some solvent (mainly water). Similarly, aqueous solutions of biologically important lipids exhibit lyotropic liquid crystal structure. Figure 1.19 may also be regarded as depicting some possible forms of lipid–water systems. The small circles represent the hydrophilic polar head-groups of the lipid molecules, while the tails are the lipophilic (i.e. hydrophobic) hydrocarbon chains. The polar groups always turn towards the aqueous phase, interacting with the polar water molecules. The hydrophobic parts, on the other hand, interact with one another by van der Waals forces. Figure 1.19*a* shows bimolecular layers of lipid molecules in section. The molecule pairs are either normal to the plane of the layer or tilted to it at an oblique angle. The diagram illustrates this latter case. The layers are separated by water, which is indicated by the horizontal shading. Figures 1.19*b* and 1.19*c* depict square and circular cross-sections of lipid rods. Many other arrangements are possible, for instance tube-like structures containing water in the inside (Figure 1.19*d*). Amphiphilic molecules may also form spheres or spherical vesicles consisting of bilayers (liposomes). These may display crystallographic structures, for instance

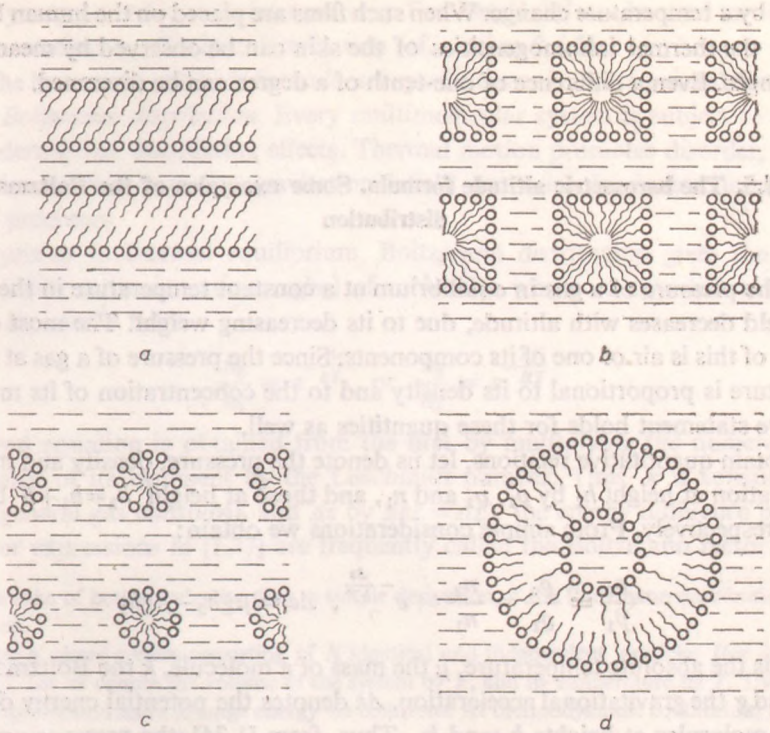

Fig. 1.19. Lyotropic systems
a: layered, *b:* prismatic, *c:* cylindrical, *d:* tubular or vesicular arrangement

face-centred or body-centred lattices. Figures 1.19*c* and 1.19*d* show structures of spherical symmetry.

Similar structures are sometimes also formed in the reverse sense, since the lipid and aqueous phases may exchange roles: in a lipid medium, layers and threads containing water molecules are formed. The basic condition for the development of the various structures in these cases too is the interaction between the ionic groups of the lipid and polar water molecules.

Liquid crystals have increased in importance recently. Reference has already been made to their biological aspects in connection with lyotropic systems. Further details will be given in section 1.5.5. Here, only two characteristic properties of thermotropic liquid crystals, i.e. *electro-optical* and *thermo-optical* properties, will be briefly described.

Due to the dipole moment of a liquid crystal, its molecular structure can be rearranged with an electric field. This rearrangement may change the optical transparency, etc. of these substances. This property can be used to transform electric signals into optical ones (cf. section 5.6.1).

A change in temperature changes the colour of a cholesteric film. The helical arrangement (the helical pitch) and consequently the reflected light can likewise be

changed by a temperature change. When such films are placed on the human body, for example the thermal inhomogeneities of the skin can be observed by means of colour changes. Even a difference of one-tenth of a degree can be discerned.

1.4.5. The barometric altitude formula. Some examples of the Boltzmann distribution

(a) The pressure of a gas in equilibrium at a constant temperature in the gravitational field decreases with altitude, due to its decreasing weight. The most common example of this is air or one of its components. Since the pressure of a gas at constant temperature is proportional to its density and to the concentration of its molecules, the above statement holds for these quantities as well.

To obtain quantitative relations, let us denote the pressure, density and molecular concentration at height h_1 by p_1, ϱ_1 and n_1, and those at height $h_2=h_1+h$ by p_2, ϱ_2 and n_2, respectively. From simple considerations we obtain:

$$\frac{p_2}{p_1} = \frac{\varrho_2}{\varrho_1} = \frac{n_2}{n_1} = e^{-\frac{\Delta\varepsilon}{kT}}, \quad \Delta\varepsilon = \mu g h_2 - \mu g h_1 \qquad [1.34]$$

where T is the absolute temperature, μ the mass of a molecule, k the Boltzmann constant, and g the gravitational acceleration. $\Delta\varepsilon$ denotes the potential energy difference between molecules at heights h_2 and h_1. Thus, from [1.34], the pressure and consequently the density and molecular concentration of a gas will decrease exponentially with altitude. As the relation is applicable to the atmosphere of the Earth, it is called the *barometric altitude formula.*

To derive [1.34], let us consider a thin horizontal layer between heights h and $h+dh$ in a vertical gas column at constant temperature, and let us denote the corresponding pressure values by p and $p+dp$, respectively. If dh is sufficiently small, the density of the gas can be considered constant; this is denoted by ϱ. The pressure on the upper boundary surface of the layer is evidently smaller than that measured at the bottom by that due to the weight of the layer; thus:

$$dp = -\varrho g\, dh \qquad [1.35]$$

The negative sign reflects the fact that a positive dh value is associated with a negative dp. Substitution of ϱ from [1.32] yields the following differential equation:

$$\frac{dp}{p} = -\frac{\mu g}{kT} dh \qquad [1.36]$$

Integration between the limits h_1, h_2 and p_1, p_2 respectively, gives [1.34].

The formula correctly describes the altitude distribution of particles suspended in air and the distribution of colloidal particles floating in liquids, so long as the temperature in the studied volume can be considered constant. Naturally, in the calculation of the potential energy of the particles both the gravitational force and the Archime-

dean buoyancy must be taken into account. Further, [1.34] can be used to determine the sedimentation equilibrium distribution (cf. section 3.5.5).

(b) The barometric altitude formula is a special case of a more general distribution law, the *Boltzmann distribution.* Every multimolecular system is subject to simultaneous ordering and disordering effects. Thermal motion promotes disorder, whereas various force fields (e.g. the gravitational field or molecular interactions) favour ordering processes.

For systems in thermal equilibrium, Boltzmann distribution gives the relative number (relative concentration; n_2/n_1) of molecules whose energy differs by $\Delta\varepsilon = \varepsilon_2 - \varepsilon_1$ in a force field:

$$\frac{n_2}{n_1} = e^{-\frac{\Delta\varepsilon}{kT}} \quad \text{or} \quad \frac{n_2}{n_1} = e^{-\frac{\Delta E}{RT}} \qquad [1.37]$$

The second equation is obtained from the first by multiplying the numerator and denominator of its exponent by the Loschmidt number. Thus, k is substituted by $kL=R$ (general gas constant), and $\Delta\varepsilon$ by $\Delta\varepsilon L=\Delta E$, the energy difference per mole. The power expressions in [1.37] are frequently called the Boltzmann factor.

For the sake of better understanding, a simple derivation of the Boltzmann distribution expression is given.

Consider a *closed* system consisting of N identical and independent particles (for instance a perfect gas). Let us denote the volume of the system by V, and its temperature by T. The particles are in random motion and exchange energy via collisions. At thermodynamic equilibrium the energy distribution of the particles does not change in time. Our task is to find this distribution, i.e. to determine the fraction of the particles which have an energy in the interval between ε and $\varepsilon+d\varepsilon$.

Before turning to more exact considerations, we may state that according to the second law of thermodynamics (cf. section 4.4) higher energy values are associated with a smaller number of particles, and vice versa.

Let us now consider the collision reaction

$$\varepsilon_1+\varepsilon_2 \rightarrow \varepsilon'_1+\varepsilon'_2$$

i.e. the process in which the collision of particles with energies ε_1 and ε_2 yields particles with energies ε'_1 and ε'_2, or conversely.

If the concentrations of particles in the energy intervals $\varepsilon_1+d\varepsilon$ and $\varepsilon_2+d\varepsilon$ are denoted by n_1 and n_2, then following the pattern of chemical equations, in unit time the reaction in one direction will take place $\vec{N}=a\times n_1\times n_2$ times, and that in the opposite direction $\overleftarrow{N}=a\times n'_1\times n'_2$ times, where a is the proportionality constant. At dynamic equilibrium:

$$\vec{N} = \overleftarrow{N}, \text{ i.e. } a\times n_1\times n_2 = a\times n'_1\times n'_2 \qquad [1.39]$$

Because of the conservation of energy:

$$\varepsilon_1+\varepsilon_2 = \varepsilon'_1+\varepsilon'_2 \qquad [1.40]$$

It follows from the independence of particles that $a=b$ and by direct substitution the function

$$n = n_0 e^{\alpha\varepsilon} \qquad [1.41a]$$

is seen to satisfy [1.39] and [1.40]. From [1.41a]

$$\frac{n_2}{n_1} = e^{\alpha(\varepsilon_2-\varepsilon_1)} \qquad 1.41b$$

[1.41b] in fact corresponds to the barometric altitude formula, i.e. to [1.37], except that the substitution

$$\alpha = -\frac{1}{kT}$$

must be performed. The negative sign is due to the fact that higher energy values are associated with smaller particle numbers, and vice versa.

Some applications of Boltzmann distribution are given below.

The *pressure of the saturated vapour* of any substance (the vapour tension, p) to a good approximation varies with temperature according to the relation

$$p \sim e^{-\frac{\Delta E}{RT}} \qquad [1.42]$$

Here ΔE is the heat of evaporation (related to 1 mole). This is the energy difference of 1 mole gas and 1 mole condensed substance in thermodynamic equilibrium at temperature T.

The *rate of a chemical reaction* is proportional to the number of activated molecules whose energy is sufficiently high for them to react with each other. In *thermal activation* the concentration c^* of molecules with sufficient excess energy, the *activation energy*, satisfies the relation

$$c^* \sim e^{-\frac{\Delta E}{RT}} \qquad [1.43]$$

where ΔE is the activation energy related to 1 mole. Since the reaction rate is proportional to the number of activated molecules, the *rate constant* k will be

$$k \sim e^{-\frac{\Delta E}{RT}} \qquad [1.44]$$

[1.44] also demonstrates that the rate of a chemical reaction increases exponentially with increasing temperature.

It follows from the above relations that the temperature-dependence of the *equilibrium constant of a chemical reaction* ($K = \vec{k}/\overleftarrow{k}$) is given by

$$K \sim e^{-\frac{\Delta H}{RT}} \qquad [1.45]$$

where ΔH is the heat of reaction ($\Delta H = \vec{\Delta E} - \overleftarrow{\Delta E}$). ΔH is negative in exothermic reactions, and consequently for this reaction type K decreases with increasing temperature. For endothermic reactions, on the other hand, K increases with increasing temperature.

Other applications of Boltzmann distribution are the Maxwellian velocity distribution, and relation [1.33] for the concentration of Schottky defects, and we shall meet it again in connection with the temperature-dependence of the concentration of thermal defects in macromolecules (cf. sections 1.5.2–1.5.5).

1.4.6. The electronic structure of solids (macromolecules). Energy band model

In both individual atoms and simple molecules, the *bound* electrons are localized in separated, well-defined energy levels, followed by the continuum, the energy region occupied by electrons removed from the atoms (*free* electrons) (cf. the energy level system of the hydrogen atom in Fig. 1.3). The situation is different for systems consisting of many atoms. Crystals, with their periodic structures, are the most easily accessible of many-atomic systems for various, detailed investigations, and a considerable amount of knowledge has therefore accumulated on the physical properties of crystals. The methods developed for crystals can be successfully applied to establish the electronic structure of biologically important macromolecules, since periodic crystals are to a first approximation good models of aperiodic macromolecules (cf. sections 1.5.2–1.5.5).

Energy bands. As a result of the interactions between the components, the electrons of a complex system are not strictly confined to individual levels, though they cannot move completely freely either. The reason for this is that, because of the interactions between the components, the electrons, mainly the valence electrons, may be exchanged. However, the electrons are not free either, since they move in the electric field of the other components, and consequently, in connection with the electronic structure of solids and macromolecules, one cannot speak of the energy levels of a single component, but of a system of energy levels. In this case, *energy bands* are formed instead of sharp energy levels (Fig. 1.20). If the system consists of N atoms forming a crystal lattice, the individual atomic electron states are split into N levels, which appears as the broadening of N discrete levels. The electrons of the system can exist only in states permitted by the broadened levels. The energy bands are the broader, the stronger the interactions between the components of the system. The lower bands are usually narrower than the higher ones, which may become so broad that the bands in this region overlap.

In the ground state the electrons occupy the lowest energy states and are excited to a higher band only by some energy uptake (e.g. light absorption, or collision with high-energy particles). However, the Pauli exclusion principle is valid for these systems too and limits the number of electrons occupying one band. Consequently, only a fraction of the electrons can be found in the lowest band, and some of the electrons are in a higher band. If this band is also filled, further electrons will occupy an even higher band, and so on. Figure 1.21 demonstrates the various possibilities. Figure 1.21*a* depicts the case where the uppermost band is only partially filled. Figure 1.21*b* shows the case when this band too is completely filled, and no partially filled band exists. In Fig. 1.21*c* a completely empty band overlaps a completely filled band. This case is similar to that shown in diagram *a*, since only a partially filled band results from the overlapping.

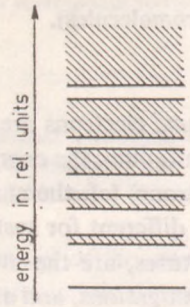

Fig. 1.20. The electron energy band system in a crystal

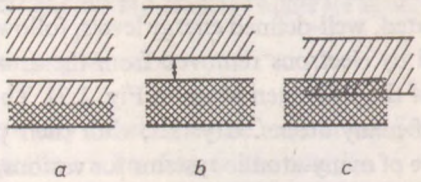

Fig. 1.21. Positions of empty, partially occupied and occupied energy bands. The occupied parts are indicated by cross-hatched lines

Cases *a* and *c* are essentially different from case *b*; in cases *a* and *c* the electrons may take up any arbitrarily small energy, whereas in case *b* the uptake of energies smaller than a certain energy limit is forbidden. The energy limit is determined by the energy required to promote an electron from the top of the highest filled band into the bottom level of the lowest empty band. This is indicated by the double arrow in the diagram. Many optical and electric properties can be easily explained on the basis of these diagrams. Only a few examples are given below.

Examples of the application of the band model. 1. Quantum mechanical calculations indicate that the energy bands of metals which are good electronic conductors can be represented by Figs 1.21*a* or 1.21*c*, whereas the band systems of insulators and semiconductors follow the scheme given in Fig. 1.21*b*. Case *a* is observed for the alkali metals, and case *c* for other metals; the band system of e.g. the insulator NaCl and the semiconductor Ge is similar to case *b*.

Electronic conduction occurs only if the electrons can take up energy from the surrounding electric field. (The result will be accelerated electron motion within the metal, whereby the electrons lose their energy by collision with the atoms, though subsequently they are again accelerated, and so on.) This type of process can occur only in cases *a* and *c*, when the electrons in the partly empty band are gradually promoted to higher levels, followed by their dropping to a lower level. This process is then repeated. It is clear that no such possibility exists in case *b*, which demonstrates the situation in insulators and semiconductors. In case *b* the electrons can absorb energy from the surrounding electric field only if they are previously somehow transferred from the filled band into an empty band. It has been observed that this transition may occur by light absorption (photoconductivity), for instance, or on bombardment of the crystal with high-energy particles. Collisions due to the thermal motion of the atoms may also promote electrons into an empty band. According to theoretical considerations but in good agreement with experience too, for the number of these electrons (n) the expression

$$n \sim e^{-\frac{\Delta\varepsilon}{2kT}} \qquad [1.46]$$

is valid, where $\Delta\varepsilon$ is the energy difference, the "gap" between the two bands i.e. the width of the *forbidden* band, T is the temperature and k the Boltzmann constant. At electronic conduction [1.46] plays a decisive role in the dependence of the electric conductivity on the width of the gap and the temperature. Thus the larger is $\Delta\varepsilon$ the better insulator is the substance and vice versa, and with rising temperature the insulating power decreases rapidly, the conductivity increases. The value of $\Delta\varepsilon$ for insulators is of the order of magnitude of eV, for semiconductors a tenths of eV or even smaller. (Semiconductors will be discussed in detail in point 3.)

2. A well-known *optical* property of insulators is their transparency in the visible range, whereas they absorb strongly in the UV and IR ranges. *Ultraviolet absorption* is related to electronic transitions, whereas *infrared absorption* is due to the vibrational excitation of the atoms, and the rotational excitation of atom groups. In the present case we consider only the possibilities of electronic transitions, i.e. we should like to explain the transparency of insulators in the visible range, and their absorption in the ultraviolet optical range. Consider Fig. 1.21*b*. Biologically important macromolecules behave in very much the same way (cf. sections 1.5.2–1.5.5). The figure shows that electrons can be excited only by high-energy photons which can raise the electrons from the uppermost filled band into the lowest empty band. It has already been mentioned that insulators require an energy of a few eV, which corresponds to the energy of the ultraviolet photons.

Metals are non-transparent in the whole optical range. This experimental fact can easily be explained; since the free electrons in the metal can take up any energy, they absorb throughout the total spectral range. The high reflectivity of the metals is connected with the same fact.

3. The electrical conduction of *semiconductors* playing an essential role in electronics can also be discussed quite easily in terms of the band model as it was already mentioned (Fig. 1.21*b*). First let us consider *intrinsic semiconductors*. These are substances where the width of the forbidden band is small enough for the electric conductivity to be sufficiently high even at room temperature. The current consists of two parts. One is created by the motion of electrons promoted into the band above the forbidden band and moved by the electric field. This originally empty band is therefore called the *conduction band*. The other part of the current is the result of a new situation in the band below the forbidden band. This is the *valence band*, since it accommodates the electrons responsible for the chemical binding. The electrons removed into the conduction band leave *holes* (defect electrons) in the valence band, which means that this band is no longer filled, and the electrons left in the valence band can take up energy from the surrounding electric field. This part of the current is the *hole current*, and the conduction is called *hole conduction*. This may be explained in the following way. The holes are created at the interatomic bonds, and they move

randomly among the atoms in the same way as the electrons split off from the bonds. The displacement of a hole can be conceived most simply in that a valence electron from its surroundings occupies the position of the hole, which in turn is shifted to the original position of the electron. This step may be repeated, with the consequence that the holes migrate in every direction within the substance. The electric field induces ordered motion, and this is superimposed on the random shifts and produces the hole current. The holes are set in motion by the electric field and drift in the opposite direction to the electrons; the holes thus behave as positive-charge carriers.

If a crystal contains foreign atoms, the force field and consequently the energy level system will be changed in the vicinity of these atoms. The situation is particularly interesting if the foreign atoms incorporated into the crystal produce free charge-carriers. Such crystals are called *doped* or *impurity semiconductors*. The foreign doping atoms either provide electrons and are called *donors*, or capture electrons in which case they are called *acceptors*. The donors increase the concentration of the electrons in the conduction band, whereas the acceptors increase the concentration of the holes in the valence band. If the donors predominate, the current is mainly due to electrons, i.e. negative charge-carriers; this is *n-type* conduction, and the crystal is called an *n-type semiconductor*. With acceptors, the current is mainly due to holes, i.e. to positive charge-carriers. In this case *p-type conduction* is involved, and the crystal is a *p-type semiconductor*. For instance, a germanium crystal doped with pentavalent arsenic is an *n*-type, and one doped with trivalent indium is a *p*-type semiconductor.

Figures 1.22 and 1.23 depict not only the band system characteristic of the basic material, but also the energy levels produced by the incorporated foreign atoms. These specific levels, however, are localized in the vicinity of the dopants and are indicated in the diagrams by short bars. The fact that the donors release electrons more easily than the basic material implies according to the band model that the highest filled donor level lies considerably nearer to the conduction band than the filled valence band of the basic material. The electrons promoted from the donor levels into the conduction band leave behind holes but, since the donor levels are

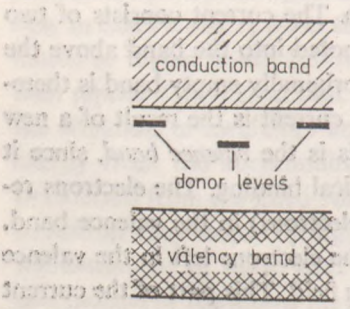

Fig. 1.22. The energy band system of an *n*-type semiconductor

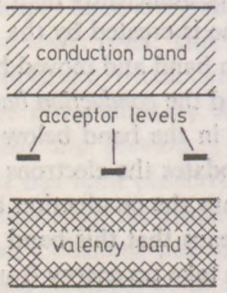

Fig. 1.23. The energy band system of a *p*-type semiconductor

localized, the holes also become localized and do not participate in the conduction. The acceptors, on the other hand, accept electrons readily, since the empty energy level of the acceptors lies close to the valence band. The electrons promoted from the valence band into the acceptor level do not participate in the conduction, and the conducting charge carriers are the positive holes left behind in the valence band.

1.4.7. Energy propagation in crystals (macromolecules)

1. Lattice vibrations (phonons). The components of an atomic system, for instance a crystal, are never at rest; they vibrate (and rotate) around their equilibrium positions. Since forces act between the components, they do not vibrate independently of one another. This is similar to a system of balls situated at equal distances and connected by elastic springs. This chain produces standing waves. The situation is much the same in crystals, though more complicated, because the atomic arrangement is three-dimensional and the vibrations propagate in every direction. The wavelength of a standing wave in a crystal may assume numerous, but only discrete values, since the only waves leading to a stationary state are those which have nodal surfaces on the boundary of the crystal. The longest wavelength is determined by the dimension of the crystal, and the shortest by its lattice constant.

Even in a given crystal, two types of lattice vibrations may exist. One is a *simple elastic acoustic vibration*, and the other type is the so called *optical vibration*. The latter is a vibration accompanied by a change in the electric dipole moment; in contrast, the acoustic wave is due to a simple elastic oscillation. The dipole vibrations are involved in the phenomenon that the crystal can emit electromagnetic radiation (infrared light) due to lattice vibrations.

In the course of their propagation the lattice vibrations amplify each other at certain places in the crystal, and attenuate each other elsewhere; further, the vibrations are scattered by lattice defects and reflected from internal boundaries. All these effects result in a complex, continually changing situation, even if the system is otherwise stationary. It follows that a crystal or a macromolecule is a coherent entity and that even in the most stable systems continuous processes, changes and transformations are going on.

Experimental evidence indicates that, similarly as for electromagnetic waves, in many cases corpuscular properties can be attributed to the propagation of lattice vibrations. In just the same way as light quanta lattice vibration quanta, called *acoustic quanta* or *phonons*, can be conceived. This name relates to the fact that the propagation of lattice vibrations is similar to the propagation of acoustic vibrations. The motion of the lattice elements forming the crystal can be described by phonons with various wavelengths, energies, polarizations, etc. It may also be said that there is a phonon field within the crystal, and on heating, for instance, the total phonon energy increases, whereas on cooling it decreases. Every external effect which perturbs the atomic motion produces new phonons which propagate in the system; the perturbation changes the phonon spectrum of the system.

2. Electrons, defect electrons, excitons. If a crystal takes up energy, not only the lattice vibrations (the phonon field), but also the electron states of the crystal may change. Such changes were discussed in the previous section in connection with the energy band model. Let us recall, for instance, those processes in which valence electrons of insulators or semiconductors were promoted to the conduction band by light absorption, whereby defect electrons were produced in the valence band. The electrons and holes migrate separately in the crystal for some time, until their recombination. The energy liberated by the recombination may be transformed into photon emission (luminescence), a rather rare occurrence in complex systems at room temperature. More frequently, the recombination energy is transformed into lattice vibration, i.e. phonons are produced.

From experience it may be assumed that in a number of cases the energy transfer is achieved by a coupled migration of the energy-carrying electrons and defect electrons, rather than by their separate motion. Such a bound electron–defect electron pair is called an *exciton*. It resembles a hydrogen atom, the defect electron taking over the role of the proton. The exciton may be in various discrete energy states.

The exciton states are created by the cloud-like expansion of the electron and hole in the crystal (cf. section 1.2.2), therefore they form energy bands extending over the whole crystal. These bands are very narrow, and are localized in the forbidden gap just below the conduction band. With increasing energy the separate bands become increasingly dense, and finally coalesce with the conduction band. The exciton dissociates if it attains a sufficiently high energy, and similarly to the case discussed earlier the electrons promoted in this way to the conduction band migrate independently from the defect electrons left in the valence band. As long as the exciton does not dissociate, it does not participate in the electric conduction. Charge can be carried only by excitons dissociated into electrons and defect electrons.

Exciton states are characteristic of ideal periodic systems. If the lattice contains defects, the expansion of the loosely coupled electron–defect electron pair is stopped, and the exciton becomes localized at the defect. This occurs in biological macromolecules, for instance in nucleic acids, which may be thought of as crystals enriched with lattice defects. As a result, the excitons in them are strongly localized. In practice the effect of excitation is found to extend only to a sequence of 3–4 bases of the macromolecule. Consequently, the fate of absorbed energy, e.g. in the form of a photochemical reaction or phonon creation, will be governed by the immediate environment of the absorption.

1.5. Structure and function

1.5.1. The properties and structure of water

The properties of water. Water is of fundamental importance not only in the development of life, but also in its maintenance. The density of water is highest at 4 °C; freezing is accompanied by a density decrease. It follows that freezing starts on the surface of lakes and seas, while below the ice cover the conditions of life in water at 4 °C are still maintained. Living organisms generally exist within fairly narrow temperature intervals. The high heat capacity of water means that this condition is ensured for organisms living in rivers, lakes or seas. In terrestrial life water is an important thermal regulator in two ways. As a result of the high water content of the tissues and the high heat capacity of water, the heat produced in the metabolic processes would increase the temperature of the body only slowly, even without thermal regulation. However, due to the high evaporation heat of water the superfluous heat is easily lost. The considerable surface tension of water plays an important role in the formation of the lipid and protein layers of the cell membranes. Further, since water is a good solvent for many inorganic and organic compounds, it is an excellent medium for biochemical reactions.

The structure of water. The properties of water are natural consequences of its structure. The water molecules are interconnected by hydrogen bonds in both the liquid and the solid state. Figure 1.24*a, b* shows the three-dimensional structure of ice. Every O atom occupies the centre of a nearly regular tetrahedron, and the adjacent O atoms occupy the vertices of the tetrahedron. For clarity, diagram *b* shows an arbitrary O atom and its environment. The H atoms between the O atoms are situated so that four H atoms are linked to one O atom by two covalent and two hydrogen bonds.

The melting of solids (including ice) involves a gradual splitting of the molecular bonds and an increase in the number of lattice defects (vacancies). In liquids the molecular order is restricted to much smaller volumes than in the solid state. With increasing temperature the number of defects increases, i.e. the order diminishes further. Simultaneously with the melting processes, ice undergoes structural transformation. The new structure consists of pentagonal dodecahedron units, as demonstrated in Fig. 1.25. These units are connected by further water molecules in such a way that one water molecule occupies the centre *(clathrate structure)*. It is important that in this new structure the water molecules are more closely packed than in ice. All these processes together lead to a characteristic density change. The decrease of the long-range order and the increase in the vacancy concentration result in a density decrease. On the other hand, the change in the tetrahedral structure increases the density. This latter process is already apparent on melting, and is most marked at 4 °C. The factors causing a decrease in the density of water predominate only at higher temperatures.

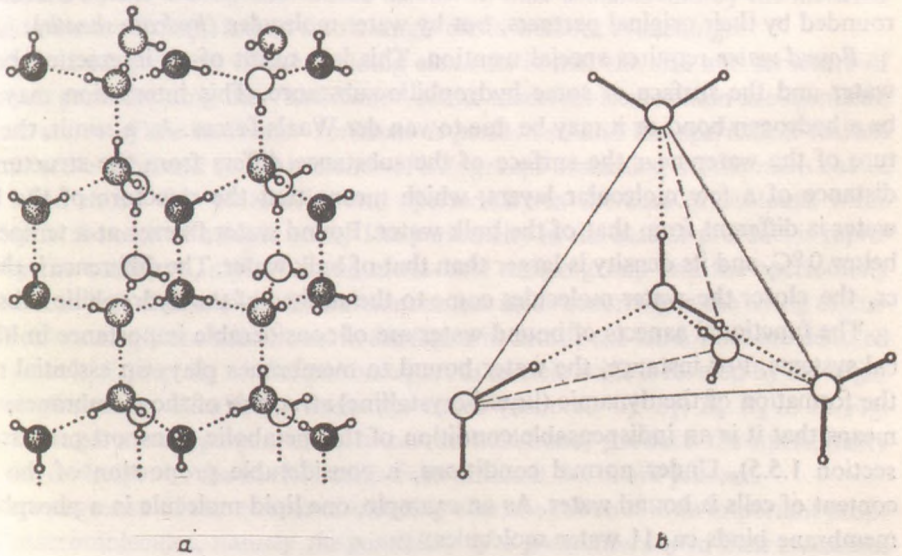

Fig. 1.24. The structure of ice
The larger circles denote oxygen atoms and the smaller ones hydrogen atoms. The covalent bonds are indicated by continuous lines and the hydrogen bonds by dotted lines

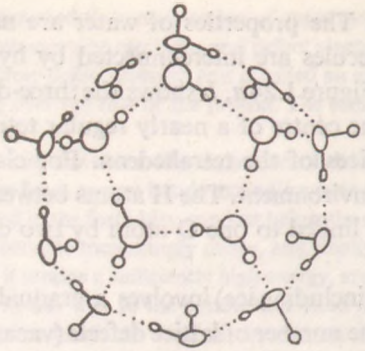

Fig. 1.25. The clathrate structure of water

It follows that an increase in temperature is accompanied by an increase in the mean kinetic energy of the molecules, together with the progressive splitting of the hydrogen bonds. This latter process requires a considerable amount of energy, which explains the high specific heat of water. The energy of one hydrogen bond in water is about 0.2 eV, which means that a single water molecule is bound with an energy of about 0.4 eV ($\sim$40 kJ/mol). The large values of the evaporation heat and surface tension are also explained by these relatively strong bonds.

The good solvent power of water is due to the relatively large dipole moment of water molecules. These weaken the bonds between the ions (atoms) in crystals by forcing apart the opposite charges, and this enables the water molecules to become wedged in between the lattice elements. The individual ions are then no longer surrounded by their original partners, but by water molecules *(hydrate sheath)*.

Bound water requires special mention. This is a result of an interaction between water and the surface of some hydrophilic substance. This interaction may either be a hydrogen bond or it may be due to van der Waals forces. As a result, the structure of the water near the surface of the substance differs from the structure at a distance of a few molecular layers, which means that the structure of the bound water is different from that of the bulk water. Bound water freezes at a temperature below 0 °C, and its density is larger than that of bulk water. The difference is the larger, the closer the water molecules come to the surface of the hydrophilic substance.

The functional aspects of bound water are of considerable importance in biological systems. For instance, the water bound to membranes plays an essential role in the formation of the dynamic (liquid-crystalline) structure of the membranes, which means that it is an indispensable condition of the metabolic transport processes (cf. section 1.5.5). Under normal conditions, a considerable proportion of the water content of cells is bound water. As an example, one lipid molecule in a phospholipid membrane binds ca. 11 water molecules.

1.5.2. Common features in the structure of macromolecules

The order of magnitude of the mass of biological macromolecules is in general from ten thousand to million dalton. All life processes are related to these molecules, and take place by their aid, are mediated by them. The *nucleic acids*, representing the genetic material of cells, the enzyme and structural *proteins* (hemoglobin, myosin, collagen, etc.), the storing and structural *carbohydrates* (starch, glycogen, chitin, cellulose, etc.) all belong to these molecules.

Though the chemical structure of these molecules is different, common structural features can be found in them, which play a role in the formation of their special properties and characteristic functions. In the following we deal exactly with these common features. Two such structural features are emphasized:

– their building up from subunits,

– the role of low-energy bonds in the maintenance of higher order structures.

The subunits as building elements are joined to form chain-like molecules by covalent bonds. The bonds are usually formed by water release. In the case of proteins the building elements are *amino acids*, and the covalent bonds forming between them, the peptide bonds, produce the so-called polypeptide chains. In the case of nucleic acids the building elements are *nucleotides*, and the polynucleotide chain is formed by the phosphate-ester bonds between them. The subunits of structural and reserve carbohydrates are various *monosaccharides*, they are bound into a chain by the glycoside bond between the carbon atoms 1 and 4, or 1 and 6.

Further on only proteins and nucleic acids will be dealt with in detail. They are characterized by *the strictly determined number of their subunits* and by the fact that the monomers are usually linked into a single chain without branching.

An important property of the building elements is that the size and structure of the subunit parts forming the "backbone" of the macromolecule chain are identical, i.e. the *structure of the molecular backbone is periodical*, and the *aperiodical* feature of the molecule is related to the presence of *side groups* branching off the main chain. Figure 1.26*a* shows this periodicity and aperiodicity in the case of proteins, while Fig. 1.26*b* in the case of nucleic acids. The periodicity in the case of proteins is represented by the carboxyl-, alpha carbon atom- and amino-group and the aperiodicity by the various side chains of the 20 different amino acids occurring in the living organisms. The former groups are shown in the figure in detail; the latter ones are denoted by the symbols R_1, R_2, R_3 ... The periodic part of nucleic acids is formed by the sugar (pentose) phosphate backbone, while the bases (denoted by B_1, B_2 B_3 ... in Fig. 1.26*b*), oriented nearly perpendicularly to the backbone, produce the aperiodicity within the molecule. In the nucleic acids four different bases are present.

The consequence of the system of building element-subunits is an important property of macromolecules, namely *the possibility of a great diversity* in their structure. This means that in the case of a chain consisting of a determined finite number of elements a high number of various polypeptide and polynucleotide chains can be

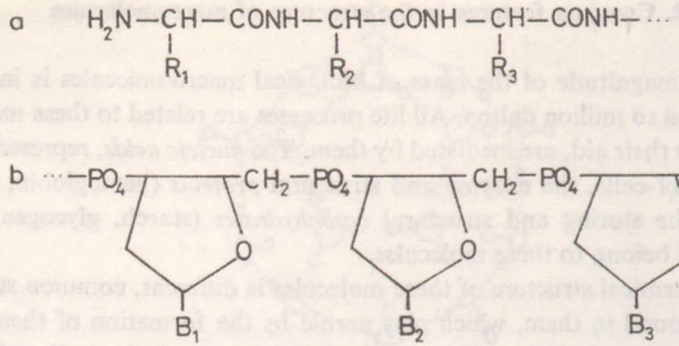

Fig. 1.26. The subunit structure of proteins (*a*) and nucleic acids (*b*)

arranged from the 20 different amino acids and the four types of nucleotides present in biological systems. In other words: since the primary structure of proteins and nucleic acids is given by the sequence of amino acids and nucleotide bases, in the case of a fixed chain length a great number of primary molecular structures can be constructed from comparatively few building elements. As an example let us consider a polynucleotide chain containing 10^6 nucleotides. The question is the following: in how many different ways can this chain be constructed from the four nucleotide bases? The calculation can be easily carried out with the following conditions:

– one of the four nucleotides can be selected to each site of the polynucleotide chain in a random way;

– the selection of each base is independent from the other bases.

It can be proved directly that in this case 4^{10^6} different base sequences are possible since for a DNA molecule constructed from k number of chain-loops the number of possible base sequences is given by the number of arrangements with repetitions of k-th order and this is 4^k. – In the case of proteins the number of elements is 20 and the order of the arrangement is determined by the number of peptide groups, i.e also by the chain length.

The great number of possible arrangements forms the basis of the *great diversity*, found in nature with respect to both genetic material and proteins. The *great quantity of information* stored in these macromolecules is also related to the great variety of the primary structure of nucleic acids and proteins. This information can be estimated in the following way. In the case of a DNA molecule containing k number of nucleotides the realization of *one selected sequence* from the 4^k different possibilities has a *probability* of $1/4^k$. From this it follows that the *uncertainty* concerning the base sequence of the chain is $\log 4^k$, which is equivalent to the statement that the average information content of the molecule is $2k$ bit (cf. section 7.1). If according to our

previous example $k = 10^6$, the average information stored by the molecule is 2×10^6 bit.[3]

The *replaceable* or *changeable character* of the building elements is also the consequence of the construction of macromolecules from subunit systems, since the incidentally defective subunits can be removed any time and replaced by a perfect one. This feature has manifold *functional significance*, for example it ensures the possibility of internal control in the elimination of the consequences of damages caused by external effects (e.g., radiation, chemicals) as well. – With respect to DNA we refer to the functioning of repair mechanisms which ensure that the genetic information stored in the nucleic acid is constant, and the change of a single nucleotide may lead to the variation of a biological function through point mutation. – In the case of proteins: the proteins performing the same function have the same amino acid sequence in the various species, but there is a different amino acid in the chain which is related just to species specificity. As an example let us consider human and horse insulin molecules. In the so-called *A* chain of the two molecules 20 amino acids and their sequence are identical, only the ninth member of the chain is different: in human insulin it is serine, in horse insulin glycine.

The primary structure formed by means of covalent bonds determines the secondary and higher-order structure of the macromolecule as well. In a given environment (pH, ion concentration, temperature, etc.) the chains assume a definite spatial arrangement, while within and between the chains *low-energy bonds* are forming which stabilize the structure. In this stabilization hydrogen bonds, ionic and van der Waals bonds play a role (cf. section 1.3.2).

The energy of a weaker bond is only between one tenth and one hundredth of an electronvolt, thus the bond can be broken thermally as well. However, due to the vast number of bonds the molecule may be stabilized. The fact is also important that in this way some bonds may be broken, some may be formed. This means a certain degree of *dynamism*, which in turn may result in the flexibility of the macromolecule and in the local rearrangement of its structure influencing its function.

At the same time the joint effect of the many bonds — as it was mentioned — ensures the *stability of the whole molecule*. As an example again the DNA molecule consisting of 10^6 nucleotides and only the hydrogen bonds formed in it will be mentioned.

The energy of a hydrogen bond can be taken to be 0.1 electronvolt, the thermal energy at 37 °C (0.02–0.03 eV) is comparable with it. Considering that in the DNA molecule the number of H-bonds and nucleotides is roughly equal, in 1 mol DNA a total energy of 10^7 kJ (!) maintains the higher structure of the molecules.

[3] In reality a smaller number is involved, as it is certain that the second condition does not hold.

1.5.3. Structure and some properties of proteins

Proteins are essential constituents of living cells, accounting for more than half (e.g. about 60% for bacteria) of the dry weight of the cells. Proteins display catalytic and contractile properties, and they also act as supports, provide protecting functions, participate in transport processes, and are basic substances of antibodies and certain hormones. For instance, one bacterial cell consists of approximately 1000 different protein molecules performing a large variety of functions. The multiple functions of proteins are connected with the nature, the number and the sequence of their components (*primary structure*) and their spatial arrangement (*secondary* and *tertiary structure*), which in turn depends on the primary structure.

Briefly on the structure. (a) *The primary structure.* Proteins are built up from *amino acids.* The smallest proteins, peptides, contain only a few amino acid residues, whereas the larger ones contain several thousand. In the various proteins 20 different amino acids may be present in practice. Their frequencies of occurrence and their sequences differ, but are characteristic of each protein type. In neutral aqueous solution the amino acid residues behave predominantly as zwitter-ions. The dipole moment of an amino acid residue is approximately eight times larger than that of water. In an acidic medium amino acids behave as cations, and in alkaline solution as anions. Both their dipole moments and charges may be influenced by the *R* groups. Naturally, not only the individual amino acids, but also the polypeptide chains built up from the amino acid residues may have dipole moments and electric charges, which are determined by the terminal amino acid units and the *R* groups.

(b) *The secondary structure.* A polypeptide chain may have a given specific form which, though determined by the *primary structure,* may also be influenced by other circumstances, such as the hydrogen ion concentration or the temperature. In their native environment, polypeptide chains are not extended. They always assume the energetically most favourable spatial arrangement (energy minimum) associated with the primary structure and the given environment.

Besides chemical methods, X-ray diffraction has proved particularly useful in the elucidation of the conformations of proteins (cf. section 3.4.1). This method provides a check on the chemically established primary structure, while the secondary and tertiary structures can be learned only by means of X-ray structure analysis. A detailed structural examination can at present be carried out only with proteins which can be produced in crystalline form, and for which the crystallization process does not change the molecular structure. The extremely complex composition of the molecules means that the X-ray diffraction pattern is rather complicated. However, evaluation may be facilitated by special methods, such as *heavy atom substitution.* This method is based on the observation that complexes containing heavy metals or other heavy elements are bound to certain specific, well-defined sites in the molecule, which in this way become labelled. Since the heavy atoms are surrounded by a cloud of many electrons which diffract X-rays strongly, the reflexions due to this effect are well visible in the diffraction pattern. However, this method yields valuable information only if the labelling does not change the molecular configuration, and the crystal containing the labelled molecules is isomorphous with the original one.

Figure 1.27 demonstrates some specific features of peptide bonding and an extended part of a peptide chain, including the bond lengths and the bond angles. The chain is of a zigzag shape. The carbon (C′) and nitrogen atoms linked by peptide bonds are in nearly the same plane with the α-carbon and oxygen, further with the hydrogen and α-carbon atoms attached to them. This is indicated by the dashed frame in the figure. The planar arrangement of the peptide group can be accounted for by delocalized π-electrons which are responsible for the bond formation of the N−C′−O atom group in the same way as in the benzene rings of aromatic compounds. For this reason the C′−N linkage is a partial double bond, while the C′=O linkage cannot be regarded as a pure double bond; consequently, rotation around the bond axis is restricted. Free rotations are possible only around the αC−N and the C′−αC bond axes. The *secondary structures* formed by the peptide chains differ in the positions of the bond planes with respect to one another. These structures are in all cases stabilized by intra- and intermolecular hydrogen bonds.

There are several types of secondary structure. One of the most frequent is the *beta-form* (also called pleated sheet). As may be seen in Fig. 1.28*a*, the beta-type peptide chains are arranged in slightly folded layers. Figure 1.28*b* depicts part of a single layer chain, whose axis consists of the αC−C′O−NH−αC groups. The *R* groups are perpendicular to the axis and are situated alternately on opposite sides of the chain. One layer consists of several parallel chains, whose axes are shown in diagram *a* by dotted lines. The stability of the chain array is ensured by hydrogen bonds (denoted by dashed lines in diagram *b*) linking the C′=O and N−H groups with the N−H and C′=O groups of the adjacent chain. The *R* side-groups are situated between the adjacent sheets. Because of the relatively large distance between the neighbouring sheets, they can easily slide on each other.

Another frequently occurring form is the right-hand alpha-helix, whose more important features are depicted in Fig. 1.29. The peptide chains are situated along a

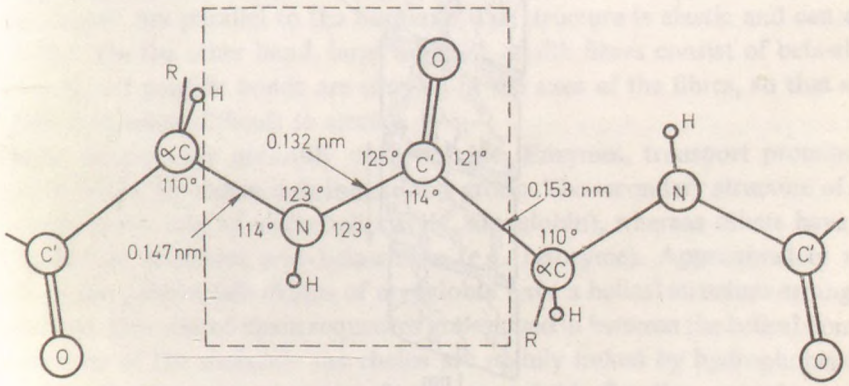

Fig. 1.27. Extended polypeptide chain

C′ denotes a carbon atom in a carboxyl group, and αC denotes a carbon atom adjacent to the carboxyl group

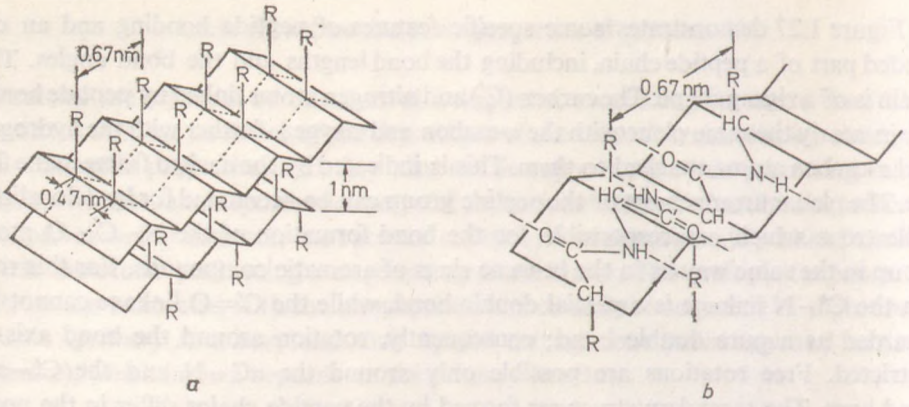

Fig. 1.28. Outline of the beta-form

helix on the lateral surface of an imaginary cylinder. The diameter of the cylinder is ca. 1.0 nm and the pitch ca. 0.54 nm and an average of 3.6 amino acids are found per turn. It follows from these data that the adjacent amino acid moieties are rotated with respect to each other by 100° around the cylinder axis, and displaced by approximately 150 pm relative to each other along the axis. The individual peptide groups are situated in tangential planes of the cylindrical shell. The *R* groups protrude from the axis. The turns of the helix are linked by hydrogen bonds between the hydrogen atom of the

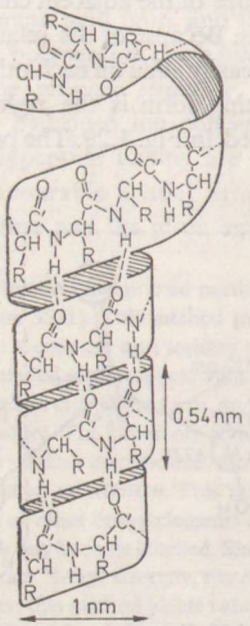

Fig. 1.29. Outline of the alpha-form

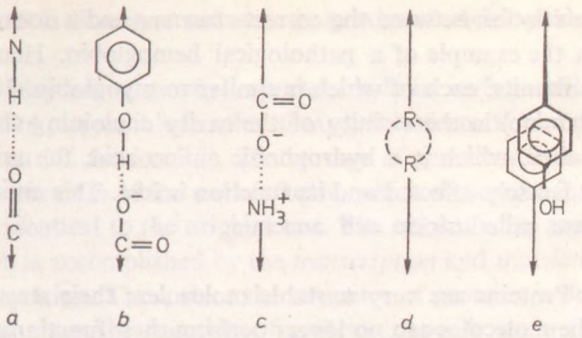

Fig. 1.30. Possible bonds between peptide chains

a: hydrogen bond between the main chains, *b:* hydrogen bond formed between side-chains *c:* electrostatic interaction (heteropolar bond) between side-chains, *d:* interaction between hydrophobic side-chains (van der Waals forces), *e:* interactions between aromatic rings of side-chains (π electron interactions)

N–H group and the oxygen of the C′=O group of the adjacent turn. Consequently, the hydrogen bonds are nearly parallel to the cylinder axis.

(c) *The higher-order structure.* The primary structure similarly determines the three-dimensional arrangement in fibrillar and globular proteins, and also the ratio of the elements of the ordered secondary structure to the elements of the disordered structure. The interactions involved in the formation and stabilization of the molecular conformation are shown in Fig. 1.30. These interactions cause the peptide chain to assume the conformation with the lowest possible energy. It is generally true that hydrophilic side-groups turn towards the molecular surface, and hydrophobic groups towards the interior of the molecule.

In structure, *keratin* (a constituent of hair, wool, feathers, etc.) is a fibrillar protein, as are muscle contractile proteins, silk, collagen, etc. Keratin contains bundles of alpha-helix type polypeptide chains arranged according to the axis of the fibre, and cross-linked by covalent disulphide bonds (formed by two sulphur atoms). Since the hydrogen bonds are parallel to the fibre axis, the structure is elastic and can easily be stretched. On the other hand, large domains of silk fibres consist of beta-sheets, and the covalent peptide bonds are situated in the axes of the fibres, so that silk is less elastic and more difficult to stretch.

Globular proteins are generally water-soluble. Enzymes, transport proteins and immunoglobulins, for instance, belong to this group. The secondary structure of some molecules consists only of alpha-helices (e.g. myoglobin), whereas others have both an alpha-helical structure and beta-sheets (e.g. lysozyme). Approximately three-quarters of the polypeptide chains of myoglobin have a helical structure arranged in eight sections. Disordered chain sequences are wedged in between the helical domains. In the interior of the molecule the chains are mainly linked by hydrophobic interactions. The heme group necessary for the myoglobin function occupies a cavity surrounded by hydrophobic amino acid residues.

The sensitive relation between the correct *structure* and a normal *function* can be demonstrated on the example of a pathological hemoglobin. Hemoglobin consists of four protein subunits, each of which is similar to myoglobin. If one hydrophilic amino acid (glutamine) in the vicinity of the cavity containing the heme group is exchanged for valine, which is a hydrophobic amino acid, the arrangement of the heme group is adversely affected and its function is lost. This amino acid exchange leads to a disease called sickle cell anaemia.

Denaturation. Proteins are very unstable molecules; their structures may easily change so that the molecules can no longer perform their functions, i.e. they become *denatured.* Structural changes may be induced, for instance, by various radiations, a temperature increase or a change in the chemical composition of the environment. The lability is due to the weakness of the bonds (hydrogen bonds and van der Waals bonds) that are of importance for maintenance of the structure. A relatively low local energy accumulation may result in the splitting of these bonds. The high degree of lability of proteins is illustrated by the fact that denaturation can be observed even at ca. 45 °C, and the probability of denaturation increases rapidly with rising temperature. The breaking of the bonds as a result of heating can be interpreted in the following way. The atoms making up a molecule are never at rest, but oscillate around the equilibrium positions (thermal motion). Their average energy is well-defined at a given temperature and increases with increasing temperature. However, the actual energy of an individual atom may be higher or lower than the average. As a consequence of the atomic interactions, higher energy may become concentrated at some bond, leading to its weakening or breaking. The resulting defects may be reverted, but a certain number of defects are always present in the molecule. According to statistical mechanics, the number of defective bonds is proportional to $e^{-\Delta\varepsilon/kT}$ (Boltzmann factor), where $\Delta\varepsilon$ is the bond energy, T is the absolute temperature and k is the Boltzmann constant. With low-energy bonds the number of defects is large because of the small value of $\Delta\varepsilon$. The above expression also reveals why the number of defects increases rapidly with increasing temperature. In reality the situation is even worse, since in the environment of defective bonds another defect may develop easier: the environment behaves as if $\Delta\varepsilon$ would be smaller. This rapidly progressing process leads to denaturation which may be observed already around 45 °C in case of proteins.

The thermal denaturation of proteins plays a decisive role in the thermal inactivation of living cells. Experience shows that the thermal denaturation kinetics of a given cell (e.g. yeast, bacterium, *Drosophila melanogaster*) coincides with the denaturation kinetics of the critical protein[4] of the cell, which means that the denaturation of this protein leads to the killing of the cell.

[4] The critical protein is the protein molecule which undergoes denaturation at the lowest temperature.

1.5.4. Structure and some properties of nucleic acids

Nucleic acids play an extremely important role in various cell functions, since these substances are responsible for the storage, transformation and transmission of genetic information (cf. section 7.1). The storage of genetic information is achieved by the *identical replication* of the nucleic acids, which means the formation of molecules completely identical to the original one. The transmission and implementation of the information is accomplished by the *transcription* and *translation* of the signals carrying the information. Thus, since the structure of proteins is determined by the nucleic acids, and since the function of proteins depends upon their structure, it may be said that the structure and function of a living cell are determined ultimately by the nucleic acids.

The great variety of living organisms is thus connected with the great variety of genetic information, which in turn depends upon the numerous variations possible in the primary structure of nucleic acid molecules.

Briefly on the structure. (a) *The chemical structure.* Depending on their chemical structures, nucleic acids can be classified into two groups: *deoxyribonucleic acids* (DNA) and *ribonucleic acids* (RNA).

Nucleic acids, as it was mentioned, are macromolecules formed by the linkage of *nucleotides.* Polynucleotide chains contain several thousand, several hundred thousand, or even several million nucleotides. Nucleotides consist of nitrogen-containing bases, phosphate groups and sugars with five carbon atoms (DNA contains 2-deoxy-D-ribose, and RNA contains D-ribose).

Four kinds of *nucleotide bases* are found in DNA: adenine (A) and guanine (G) with a purine skeleton; thymine (T) and cytosine (C) with a pyrimidine ring. In RNA the same two purine bases and cytosine occur as in DNA, but the second pyrimidine is uracil (U) instead of thymine. (U differs from T only in not containing a methyl group on carbon atom 5; cf. Fig. 1.31.) Depending upon the environment, the bases may exist in different tautomeric forms. Figure 1.31 shows the structures most frequently occurring in nature.

The different bases are not present in equal numbers in a given nucleic acid, but the double-stranded DNA and RNA contain the same number of adenine as of thymine (uracil) and as many guanine as cytosine.

(b) *Three-dimensional structure.* The first conformation successfully elucidated was that of the double-stranded DNA (Watson and Crick 1953, Wilkins et al. 1953).

The exact determination of the DNA conformation was carried out by means of X-ray diffraction (cf. section 3.4.1). Individual fibrillar molecules were investigated by the Laue method, and polycrystalline samples by the Debye–Scherrer method (Figs 1.32 and 1.33, in the Supplement). These studies were based on earlier, similar analyses of the more simple crystalline nucleotide bases and other DNA components,

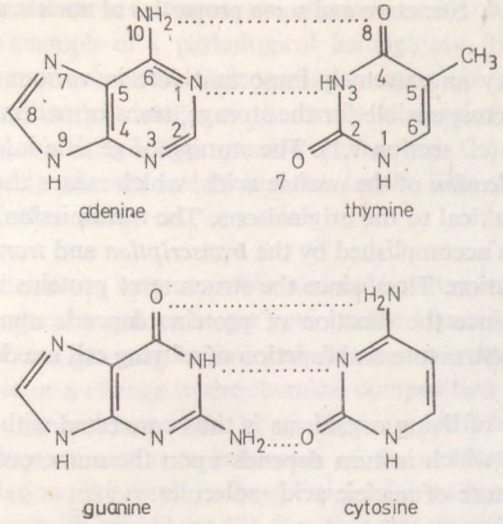

Fig. 1.31. Structural formulae of nucleotide bases

The bases on the left contain purine rings, and those on the right contain pyrimidine rings. The numbering sequences for A and T also apply to G and C, respectively. The dotted lines indicate the sites of hydrogen bond formation between the A–T and G–C base pairs in DNA

mainly with the X-ray diffraction technique. The results of these studies revealed that the DNA molecule consists of two helical polynucleotide chains (Fig. 1.34). The helices are generally right-handed. The outer parts of the chain are occupied by the sugar and the hydrophilic phosphate groups, while the hydrophobic bases are inside the double helix. The atoms of one base are nearly coplanar, and the planes

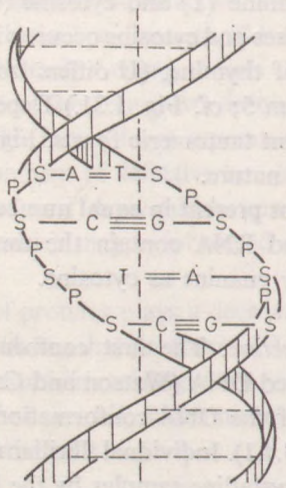

Fig. 1.34. Outline of the structure of DNA

S: sugar, *P:* phosphate, *A:* adenine, *T:* thymine, *C:* cytosine, *G:* guanine. The thin lines drawn within the helices, and the double and triple lines between the bases in the centre of the figure denote hydrogen bonds

of the bases are parallel to each other. The two helices are connected by the bases opposite each other in the individual chains. Further, opposite the positions occupied by adenine in one of the DNA chains the other chain contains thymine. Guanine is similarly always paired with cytosine. The two members of a base pair are situated in one plane, and the members of the complementary pairs are connected by hydrogen bonds: for the A–T pairs by 2 hydrogen bonds, and for the G–C pairs by 3 hydrogen bonds per pair. (Figure 1.31 shows the atom pairs forming these hydrogen bonds.) These base pairings explain the analytical chemical findings relating to the frequencies of occurrence of the bases, and also account for their spatial arrangement, since the smaller pyrimidine is always coupled with the bulkier purine.

Various forms (conformations) of the DNA molecules are known. The above findings hold for all of them, but there are differences in the fine structure, which depend upon the molecular environment (e.g. temperature, water content, hydrogen and sodium ion concentrations). The *B*-conformation occurs more frequently; its most important data are presented in Fig. 1.35*b*. The molecule contains 10 nucleotides per turn and the planes of the bases are normal to the axis of the helix. This *B*-conformation occurs in physiological aqueous solutions. In a medium with less water content a slightly different, though also right-handed conformation is found. A dried bacterium spore, for instance, contains the *A*-conformation, which may be considered an extreme case as concerns its parameters (Fig. 1.35*a*). A smaller helical pitch and a larger fibre thickness are the characteristic features of this conformation. One turn contains 11 nucleotides. In this conformation too these base pairs are parallel to each other, but the angle between them and the planes normal to the helical axis is 20°. The other DNA conformations differ from each other and the above

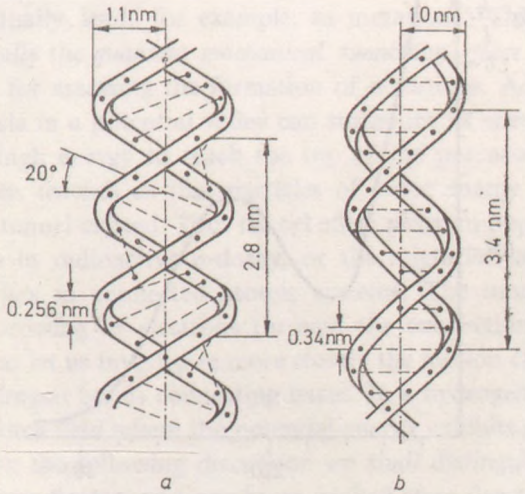

Fig. 1.35. DNA conformations and characteristic data
a: A-form, *b: B*-form

types in pitch, the angles of the bases, and the number of nucleotides per turn. (It has recently been reported that a left-handed form may also occur in some sections of DNA.)

In living organisms the conformations of nucleic acids are influenced by their interactions with proteins (structural proteins and enzymes). In this connection it should be mentioned that the double-stranded DNA occurs quite frequently as closed, circular molecules rather than open ones. In this case, the coupling of the complementary chains and hence the conformation of the nucleic acid is fixed. The structural mobility necessary for the biological functions (i.e. the conformational transitions) is provided here too by proteins (enzymes).

The double-stranded RNA may form helical conformations more or less similar to DNA, but the complementary base of adenine in this case is uracil. It often occurs that certain sections of a single RNA chain are complementary to one another, and an intrachain helical structure stabilized by hydrogen bonds is formed affecting only one detail of the molecule (e.g. transfer RNA, virus-RNA).

From the discussion so far, partial answers can now be given to the questions concerning the relation of the structure and function of the nucleic acids. Such problems are e.g. the storage, conservation, transfer and translation of the genetic information.

(c) *A few words on electronic structure.* Figure 1.36 depicts the absorption of a DNA solution. The spectrum shows that the molecules absorb in the ultraviolet range; the first absorption maximum is at 260 nm, which means that the smallest excitation energy of the DNA electron structure is about 4.8 eV. Quantum chemical methods exist for the determination of the electronic states (e.g. charge density

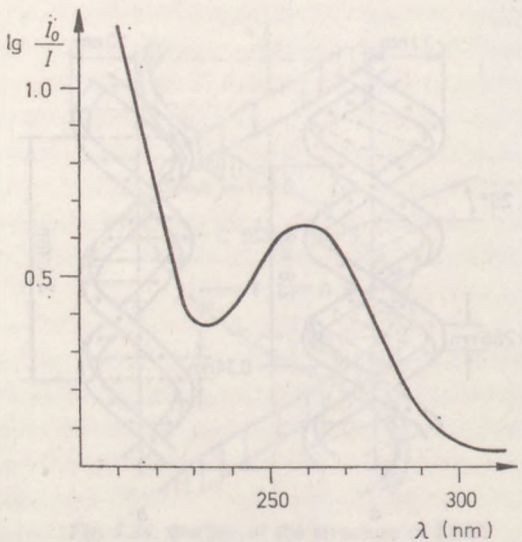

Fig. 1.36. Ultraviolet absorption of DNA obtained from a bacteriophage

characteristics for individual atoms, bond strengths and ionization energy) for the individual nucleotides. However, as yet no theoretical method is known for interpreting the total electronic configuration of the aperiodic DNA system. At any rate, the optical absorption suggests that the width of the forbidden band is about 4.8 eV.

Though the optical absorption spectrum of DNA is similar to the spectrum of the individual nucleotides making up the macromolecule, it still cannot be constructed as the sum of these component spectra. In fact, the π electrons distributed around the atoms of the bases above one another interact by van der Waals type forces (stacking interaction) both in the ground state and in the excited states generated by the absorbed UV light. These interactions are manifested in a weaker absorption than the sum of the absorptions of the individual components. For a double helix, for instance, the attenuation may amount to 30–40%. This is the *hypochromic effect*. Study of the absorption spectrum yields information not only on the primary, but also on the secondary structure, since the height of the absorption band maximum is characteristic of the strength of the stacking interaction.

Some properties. A comprehensive study of the relations between the conformation and electronic structure and the biological function of DNA molecules is in progress. Among the essential questions are the elucidation of the structural transformations leading to mutations, and the understanding of the connections between the DNA structure and the mechanism of duplication. Detailed research work is continuing to investigate the structural and functional changes due to spontaneous effects and various agents.

In this section some interesting results will be discussed.

(a) What are the reasons for the defects in the base sequence, and how do these defects eventually lead, for example, to mutations? This question has several answers; actually the *quantum mechanical tunnelling effect* may be taken as a possible departure for assessing the formation of mutations. According to classical mechanics, a particle in a potential valley can surmount its energy barrier only if it has a sufficiently high energy to reach the top of the potential barrier. Quantum mechanics, however, teaches us that particles of lower energy may also cross the barrier, as if some tunnel existed. Thus tunnel effect plays an important role in many cases, for instance in radioactive α-decay, or the migration of electrons through the potential barriers in connected atomic systems. The tunnelling process also accounts for the crossing of electrons through the connections of electric power lines. In this context let us investigate more closely the motion of hydrogen atoms or protons in the hydrogen bonds connecting bases. In a hydrogen bond the hydrogen atom moves in a force field where the potential energy exhibits minima at two positions (Fig. 1.37). In the following discussion we shall distinguish between a DNA molecule in the ground state, and one in an excited state due to UV irradiation or some other excitation. The potential curve in the first case is strongly asymmetric,

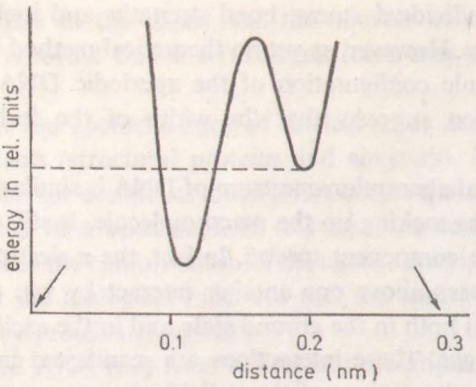

Fig. 1.37. Variation in potential energy along the distance between the pillar atoms in a hydrogen bond
The arrows indicate the positions of the pillar atoms

as shown in Fig. 1.37, but in the second case the curve is nearly symmetrical, and the depths of the two energy valleys are approximately the same. In the ground state of the molecule the hydrogen atom is localized with high probability in the deeper valley, which also means that the hydrogen atom can usually be found in the vicinity of the atom with which it was associated before formation of the hydrogen bond. The hydrogen atom can pass into the shallower valley by means of tunnelling only if it takes up energy (e.g. infrared light) to attain the level corresponding to the bottom of the shallow valley, as indicated by the dashed line in the figure. With excited molecules the situation is different, since in this case the hydrogen atom may occupy either of the two potential valleys with approximately the same probability, i.e. it may be found in the vicinity of either pillar atom, i.e. in the vicinity of either member of the base pair. The transfer of the hydrogen atom from one base to its pair implies that *tautomeric bases* different from the original ones are created. If this occurs in DNA duplication, the complementary bases corresponding to the unusual tautomeric forms appear. This process may lead to defective base sequences, which possibly results in mutation. This picture explains the experimental fact that mutations arise very rarely under ordinary circumstances, whereas they are produced with high probability by ultraviolet light or high-energy photons or particles.

(b) The fundamental and extensively studied effect of *denaturation* in biological macromolecules means the loss of the characteristic biological properties. This effect is connected with structural changes and is reminiscent of the melting of solids, since in both cases ordered structures become less ordered. As an example, mention may again be made of solutions containing DNA molecules. From experience it may be concluded that the double helices of the DNA molecules become separated first at some places, and subsequently along the whole chain, and the resulting single chains assume a less ordered coil-like form. This process may be compared to the

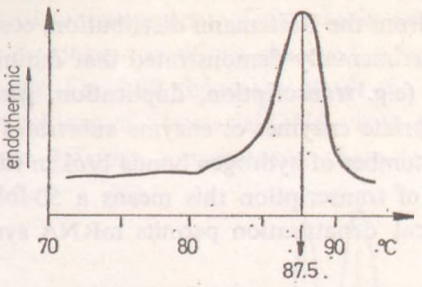

Fig. 1.38. Melting of DNA obtained from phage T7
The abscissa shows the temperature of the solution and the ordinate the quantity of heat absorbed. The melting point is also indicated

melting of solids in that it is accompanied by an energy uptake, the amount of which can be measured by microcalorimetry (Fig. 1.38). The diagram demonstrates the melting of T7-DNA as an example. The transition takes place in the temperature interval between 80 and 92 °C. The melting point is to be found roughly at the centre of this interval, at 87.5 °C. It should be stressed, however, that the interval of transition and the melting point as defined depend upon the circumstances, for instance upon the hydrogen ion and other ion concentrations of the solution containing the DNA molecules. For DNA molecules of various origins the melting point also depends on the relative quantities of the G–C and A–T base pairs. The higher the G–C content, the higher the melting point under otherwise identical conditions. For instance, at neutral pH and 0.2 mol/l KCl concentration the DNA molecule of *Diplococcus pneumoniae* with a 40% G–C content melts at ca. 85 °C, whereas the melting point of the DNA molecule of *Mycobacterium phlei* with a G–C content of ca. 75% under identical circumstances is approximately 97 °C. If a heated DNA solution is cooled at a sufficiently slow rate, the vast majority of the separated chains can recombine, and double helices are formed again. This process is called *renaturation.* While denaturation is reminiscent of the melting of crystals, renaturation rather resembles freezing; the interpretation of these processes is similar too. With increasing temperature, i.e. with increasing thermal motion of the atoms, the low energy (hydrogen, van der Waals) bonds break first, and the two helices become uncoiled. New bonds are formed between the atoms of the isolated chains, which finally results in coil-shaped molecules. The melting point is the temperature above which this coil-like structure becomes relatively frequent, whereas below it the helix form is energetically more stable.

(c) In the previous point we dealt with total denaturation, which finally results in the cessation of the biological functions. However, an important role in biological functions is attributed to *partial (local) denaturation.* Partial denaturation means the local splitting of the hydrogen bonds, which may occur spontaneously even at 37 °C. If the mean binding energy of the hydrogen bonds of the DNA molecules is

30 kJ/mol, 10^5 base pairs in a DNA molecule contain one defective hydrogen bond; this can be calculated from the Boltzmann distribution, considering thermal motion alone. It has been experimentally demonstrated that during molecular mechanisms connected with DNA (e.g. transcription, duplication, genetic recombination) the presence of the appropriate enzymes or enzyme substrates leads to a considerable increase locally in the number of hydrogen bonds broken in the environment of the interaction. In the case of transcription this means a 50-fold increase in the defect concentration. This local denaturation permits mRNA synthesis corresponding to complementarity.

Nucleic acid-protein complexes. Nucleic acids usually exist in nature in interaction with proteins, in the form of nucleic acid–protein complexes. Both DNA and RNA may form complexes; examples of the former case are chromatin, i.e. the material of the cell nucleus, and DNA-containing viruses, and examples of the latter are the cell ribosomes and the RNA-viruses.

Some of the interacting proteins exhibit enzyme activity; these take part, for example, in the transformation and implementation of the genetic information stored in the nucleic acid. Other proteins are essential in the higher-order structure of nucleic acids, thereby ensuring the information-storing function of the genetic material (mainly the DNA). Since the storage and transformation are accomplished by the interaction of one and the same nucleic acid with different proteins, this is possible only if the complexes possess a high structural mobility ensured just by the low-energy bonds (cf. section 1.3.2), i.e. even small local changes (e.g. the appearance or disappearance of some ions or molecules) may result in the dissociation of the complex and produce a new one. Deeper insight into these processes is given by an understanding of the molecular structure.

To establish the molecular structures of the complexes, almost all up-to-date methods of physical structure analysis are applied, e.g. small-angle X-ray and neutron scattering, Raman spectroscopy and UV absorption and luminescence spectroscopy (cf. sections 3.3–3.5) which yield information from various aspects on the nature of the nucleic acid–protein interaction and its consequences. Some characteristic, common structural features of DNA-containing complexes will be discussed in this section.

The inner core of the complex is a protein; the double-stranded DNA is coiled around this, forming a superhelix. Further protein molecules are attached to the outer side of the complex. The DNA conformation in the superhelix is nearly of the *B*-form (see Fig. 1.3.5*b*), but due to the interaction with the proteins it differs somewhat from it, the regular arrangement of nucleic acid bases being distorted: the distance between the base planes, the positions of the planes relative to each other and to the helix axis, etc. are changed. This indicates that the π electron interaction between the base pairs is smaller in the complexes than in *B*-form DNA (e.g. in solution). On the other hand, if the nucleic acid – protein interaction ceases partially or totally (e.g. due to slow heating or removal of the protein), a hypochromic effect can be detected in the UV absorption band of the nucleic acid, which shows that the π electron interaction between the base pairs increases and a DNA with regular *B*-conformation appears in the solution.

Another example of the behaviour of the nucleic acid–protein complexes is that heating causes a structural rearrangement of chromatin and virus nucleoproteins. The process is similar to the phase transitions observed in liquid crystals (cf. section 1.4.4). As an example of this, Fig. 1.39 shows the result of a microcalorimetric measurement on the phase transitions of a bacterial DNA-virus, phage T7. The diagram shows transitions at three different temperatures, characterized by different heat

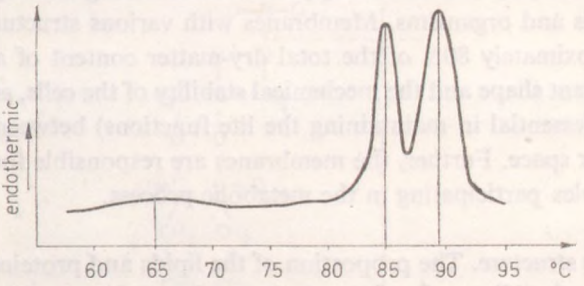

Fig. 1.39. Phase transitions of phage T7

absorptions. The difference from Fig. 1.38 is striking; this is related to the fact that we were dealing there with pure DNA and here with a nucleoprotein. From a biological aspect the transition at 65 °C is of special interest. This can be attributed to the fact that due to heating the nucleic acid–protein complex partly dissociates, and is partly transformed, and the superhelical structure of the DNA is destroyed. The process may take place under *in vivo* conditions too, though in that case it results not from a temperature increase, but from a change in the physico-chemical environment. This was mentioned above in connection with the significance of local changes in the breaking of weak bonds and the formation of new ones. As a consequence of the outlined transformation, after the partial dissociation the DNA is able to interact with another protein exhibiting enzyme activity. This produces a situation favourable for the transmission of information stored in the DNA (replication, translation).

Finally, the double maximum (at 85 °C and 89 °C) should be mentioned, though it has no direct biological significance. The double maximum is also characteristic of the melting of DNA (cf. Fig. 1.38), but the process is now modified by the residual protein–DNA interaction: the DNA stabilized by the interaction undergoes a phase transition at 89 °C, while in the maximum at 85 °C the conformational change of the proteins also plays a role.

1.5.5. Structure and some properties of biological membranes

The membranes of cells and of their individual cell constituents (e.g. the mitochondria) are composite systems consisting of smaller molecules, whose main components are lipids and proteins. The structure of the membranes—considering mainly their

lipid constituents—reminds of that of the macromolecules, since they are also composed of subunits, and the weak energy bonds characteristic of macromolecules are important in the preservation of the molecular structure. However, they differ from the macromolecules, since the subunits—in the present case the lipid molecules—are not connected into chains (cf. section 1.5.2), instead they form layers, whose monotony is disrupted by larger protein molecules.

The membranes play a determining role in maintaining and regulating the functions of the cells and organisms. Membranes with various structures and functions constitute approximately 80% of the total dry-matter content of animal cells; they ensure the constant shape and the mechanical stability of the cells, and the concentration difference (essential in maintaining the life functions) between the intracellular and extracellular space. Further, the membranes are responsible for the transport of ions and molecules participating in the metabolic process.

Briefly on the structure. The proportion of the lipids and proteins constituting the membranes, though different for the various membrane types, may be regarded constant within given genetic and physiological conditions. However, in case of a lasting change of the external conditions (e.g. cooling) the lipid composition changes (cold adaptation).

The majority of the *membrane lipids* are phospholipids, which consist of a polar head-group and in most cases of two parallel apolar hydrocarbon chains containing 14–18 carbon atoms per chain. The hydrocarbon chains may be saturated, or may contain one or more double bonds. Figure 1.40 depicts the phosphatidylcholine (lecithin) molecule, which is a component of every biological membrane. The molecule consists of a glycerine backbone (*a*), a choline phosphate group (*b*), and two palmitic acids (16 carbon atoms) linked with the hydroxyl groups of glycerine. Besides the polar phospholipids (e.g. phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine), the various membranes contain among others considerable but different amounts of apolar cholesterol, which is important in the formation of the membrane structure. The chemical composition allows the lipid molecules to form van der Waals interactions with one another and also with other molecules.

The quantity and amino acid composition of the *membrane proteins* also vary considerably in the different cell types. The protein/lipid ratio ranges between 0.3 (myelin) and 3.0 (bacterial membranes), but in most cases it may be taken as 1.0 (erythrocytes, the outer membranes of mitochondria, etc.). Similarly, membrane proteins with various functions contain polar, weakly polar and apolar amino acids in approximately the same proportions. Accordingly, the proteins may develop stronger (electrostatic) or weaker (van der Waals type) interactions with one another and with their environment.

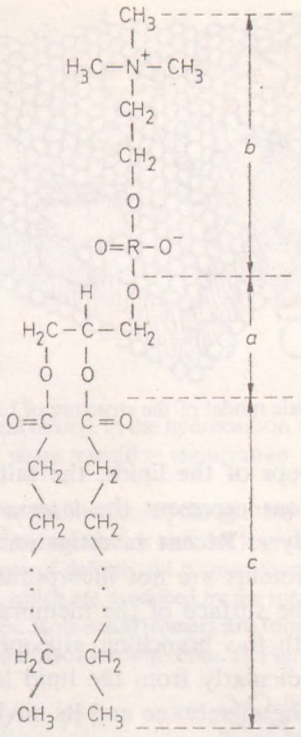

Fig. 1.40. Structural formula of dipalmitoyl phosphatidylcholine

The structure of membranes is mainly determined by the dual character (polar and apolar) of their lipid and protein content, i.e. their amphiphilic nature. In aqueous medium the molecules are ordered so that the polar groups turn towards the aqueous phase and get into electrostatic interaction with one another and with the dipolar water molecules. The hydrophobic parts are linked by van der Waals forces inside the membrane. As pointed out in connection with liquid crystals (cf. section 1.4.4), plane and bent lipid bilayers are to be found with high probability among the thermodynamically possible configurations of the lipid–water lyotropic systems. The structural basis of the membranes is the lipid bilayer, in which (as mentioned above) the polar head-groups interact with water on the two sides of the membrane, while the hydrocarbon chains of the two layers are turned towards each other, resulting in an easily changing *liquid crystalline* structure of the membrane. This hydrophobic part is responsible, for instance, for the high electric (and diffusional) resistance and the high electric capacity of the membranes. The membrane proteins are intercalated into this "fluid" lipid layer to an extent depending upon their geometric and charge configurations, determined by their amino acid content and sequence. Some proteins may reach completely across the lipid bilayer. This arrangement called *fluid mosaic* model is shown in Fig. 1.41. The small spheres on both sides of the membrane

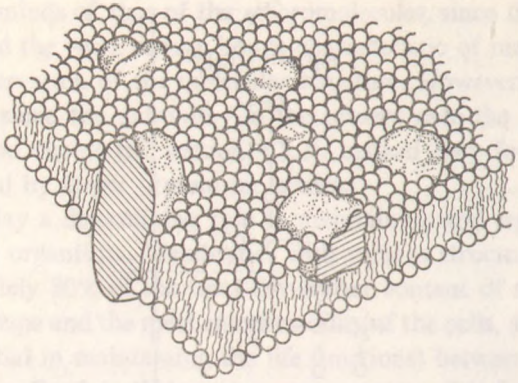

Fig. 1.41. Fluid mosaic model of the structure of biological membranes

represent the polar head-groups of the lipids, the tails represent the hydrocarbon chains, and the dashed regions represent the *integrant* proteins penetrating into, or even through the lipid layer. Recent investigations indicate that a small proportion of the membrane proteins are not incorporated into the lipid layer, and are only loosely bound to the surface of the membrane. These are the *peripheral* proteins which, together with the branching glycoproteins (hydrocarbon–protein complexes) emerging perpendicularly from the lipid layer, play an important role in the interactions between the membrane and its environment (for instance in the immune processes).

The *water layer bound* by the polar groups on the surface is also part of the membrane structure, though not much is known about its molecular configuration. Experimental results suggest that some of the surface water is strongly bound and consequently more ordered. A loosely-bound intermediate layer with dynamic configuration covers the strongly-bound water, followed by the intra- or extracellular solution. (This ordering of the water bound to the surface can be found in all biological systems.)

Some properties. Most properties of biological membranes can be attributed to the liquid-crystalline behaviour of the lipid layers. The structural transformation of the apolar phase is of fundamental importance in the function of the membrane. In straight, ordered hydrocarbon chains with *all trans* configurations, one or more breaks (gauches) appear above the *temperature of the phase transition,* resulting in a less ordered configuration (Fig. 1.42). In this process the otherwise weak bonding between the individual chains becomes even weaker, with the result that the molecules are more widely separated from one another and the number of structural defects suddenly increases.

Various types of structural defects may appear, depending upon the external effects (e.g. temperature change, pressure, electric field, drugs) modifying the membrane structure. The number and type of the structural defects are decisive in the development of the membrane functions. The defects

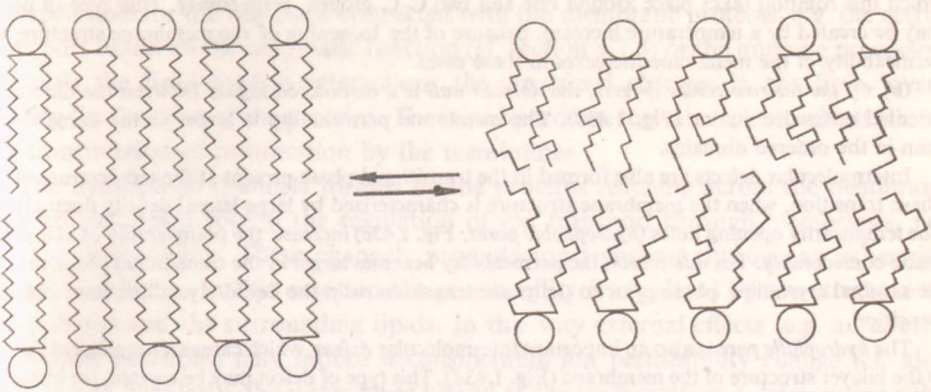

Fig. 1.42. Outline of the structural change in the hydrocarbon chains of the lipid bilayer at the phase transition temperature

determine mainly the permeability of the lipid layer, though they may be important as concerns the mechanical properties (elasticity) of the membrane and the lateral motion of the membrane components (e.g. proteins). Two basic types of defects will be mentioned:

(a) First *intramolecular defects*, which are produced by the rotational isomerization of the hydrocarbon chains of the lipid molecules. Rotational isomers are formed if the chain segment is rotated by $\pm 120°$ around a C–C bond of the hydrocarbon chain. In Fig. 1.43*a* two isomers are presented in

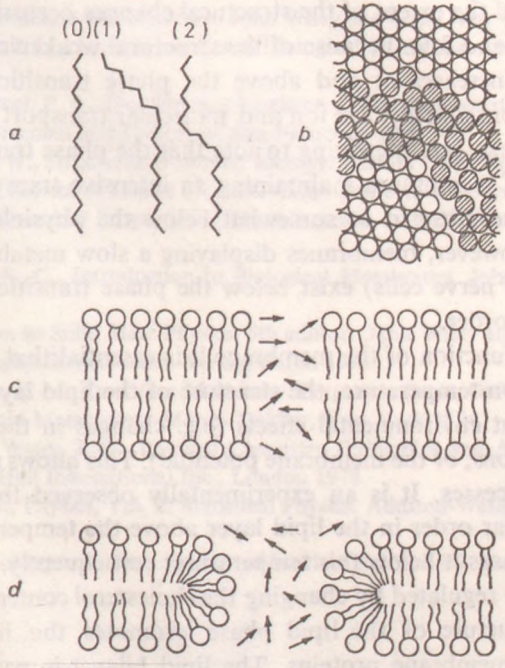

Fig. 1.43. Structural defects in lipid membranes

a: hydrocarbon chains with one (1) and two (2) rotational defects (isomers) (a perfect chain is shown on the left); *b:* lipid membrane (viewed from above) with differently oriented ordered domains and the domain wall (shaded circles); *c:* hydrophobic pore; *d:* hydrophilic pore

which this rotation takes place around one and two C–C groups, respectively. This type of defect may be created by a temperature increase. Because of the loosening of the membrane structure, the permeability of the membrane increases in these cases.

(b) *Of the intermolecular defects,* the *domain wall* is a disordered region between the differently oriented ordered domains (Fig. 1.43*b*). The membrane permeability is larger at the domain wall than in the ordered domains.

Intermolecular defects are also formed in the transitional phase present at the temperature of the phase transition, when the membrane structure is characterized by large lateral density fluctuations. The temporarily opening holes (*hydrophobic pores;* Fig. 1.43*c*) increase the permeability of the membrane considerably. For this reason the permeability becomes larger in the transitional phase than in the original crystalline phase prior to the phase transition or in the liquid-crystalline state after the transition.

The *hydrophilic pore* is also an important intermolecular defect, which causes a topological change in the bilayer structure of the membrane (Fig. 1.43*d*). This type of defect may be created, for instance, by applying a static electric field or by introducing wedge-shaped lipid molecules (e.g. lysolecithin) into the membrane. The hydrophilic pores also have an important permeability-increasing effect.

The structural changes occurring at the temperature of the phase transition can be followed well with physical structure analysis methods which are sensitive to changes in the molecular order. Primarily electron and X-ray diffraction methods, laser-Raman spectroscopy, magnetic resonance spectroscopy (ESR, NMR) and the microcalorimetric methods (e.g. DSC) are suitable for determination of the transition temperature, and the extent of the structural changes occurring at this temperature (cf. sections 3.3 and 3.5). Because of the structural weakening, the permeability of the membranes increases at and above the phase transition temperature (cf. section 4.7.2), and consequently the ion and molecular transport through the membrane will also increase. It is interesting to note that the phase transition temperature of the lipid layers of membranes maintaining an intensive transport (e.g. the mitochondrium) are found close to or somewhat below the physiological temperature. At the same time, however, membranes displaying a slow metabolism (for instance the myelin sheath of nerve cells) exist below the phase transition temperature, i.e. in a state of higher order.

In the regulating function of the membrane it is essential that, in particular close to the phase transition temperature, the structure of the lipid layer may be changed considerably by slight environmental effects (e.g. changes in the concentrations of hydrogen and other ions, or the membrane potential). This allows sensitive regulation of the transport processes. It is an experimentally observed fact that cholesterol increases the molecular order in the lipid layer above the temperature of the phase transition, and decreases it below this temperature; consequently, the permeability of the membrane can be regulated by changing the cholesterol content of the membrane.

The dynamic structure of the lipid phase promotes the finer regulation of the function of the membrane proteins. The lipid bilayer in part ensures a mobile medium for the conformational change of the integrant proteins, and in part permits the lateral motion of the proteins in the plane of the membrane. Both processes are

indispensable for the functions connected with the membrane proteins, e.g. the active transport based on the enzymatic function (cf. section 4.7.3) or the immune processes. Through the lipid–protein interactions the structural changes in the lipid layers modify the function of the proteins. The reverse process too takes place, which allows the transmission of information by the membranes.

The hydrophilic channels formed by the proteins reaching across the membrane play an important role in the regulation of ion transport through membranes, i.e. the ion permeability. These channels promote ion transport through an otherwise apolar membrane. In this case too the permeability is regulated by the structure of the proteins and the surrounding lipids. In this way external effects (e.g. an altered membrane potential) may induce a considerable ion permeability change (cf. also section 6.1). Similarly, the water layer bound at the surface plays a determining role, for by interacting with the hydrate shells of the ions and polar molecules (e.g. amino acids) it participates in the regulation of the transport of these substances.

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2. RADIATION. THE PHYSICAL BACKGROUND OF THE APPLICATION OF RADIATION IN MEDICINE

2.1. The complete electromagnetic spectrum

The electromagnetic spectrum with its ranges is shown in Fig. 2.1. Only visible light stimulates the visual receptors of the human eye. Its wavelength range is a fairly narrow one, from 400 to 800 nm. However, the concept of light includes not only visible, but also infrared (IR) and ultraviolet (UV) radiation. These three forms are collectively known as the optical interval of electromagnetic radiation. X-radiation, with an extremely short wavelength (less than 100 nm), is also called light, which expresses the fact that any electromagnetic radiation resulting from changes in state of electrons, atoms or molecules (multi-atomic systems), i.e. from processes outside atomic nucleus is called light. On the short wavelength side of the light range follows (with a considerable overlap) γ-radiation produced by nuclear processes, and on the long wavelength side the electromagnetic waves produced by the telecommunication techniques.

Nearly all properties of the telecommunication range can be interpreted by the physics of waves, whereas almost all properties of γ-radiation are rather of a corpuscular character. In the intermediate interval of the electromagnetic spectrum neither property predominates. Numerous processes may be interpreted electromagnetically, and others by means of the corpuscular character.

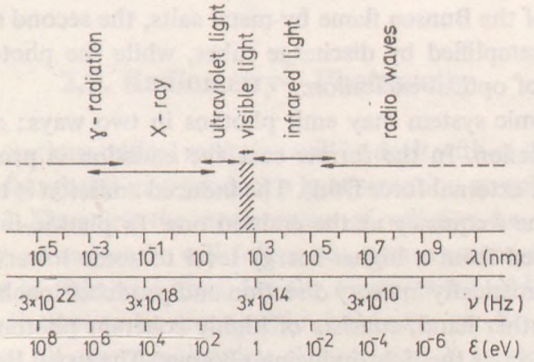

Fig. 2.1. The complete electromagnetic radiation spectrum
λ: wavelength; ν: frequency; ε: photon energy

Within the visible range the photons of various wavelengths produce different colour sensations:

400–420 nm: violet
420–490 nm: blue
490–540 nm: green
540–640 nm: yellow
640–760 nm: red.

No sharp limits exist: the transitions from one colour into another are gradual.

In the next section only the *optical range* will be discussed. X-radiation is dealt with separately, and γ-radiation is treated together with nuclear radiations.

2.2. Interaction with atomic systems

The emission and absorption of light are produced by changes in state of atomic systems or more exactly by the changes in electric dipole moment of these systems. Just these radiative transitions are allowed by the selection rules discussed earlier (sections 1.2.2 and 1.3.3). The electric dipole moment may change during electronic transitions, but also as a result of changes in the rotational or vibrational states of atoms or molecules. Light emission is connected with a change in state of optical electrons, whereas X-radiation is a consequence of a change in state of the inner shell electrons.

1. Emission. An atomic system (atoms, molecules, multi-atomic systems) may emit light only if the system possesses excess energy in relation to its ground state, i.e. if it is in an excited state. An atomic system may be excited in different ways: thermally, electrically or optically. An example of the first case (thermal excitation) is the coloration of the Bunsen flame by metal salts, the second case (electrical excitation) may be exemplified by discharge tubes, while the photosynthesis of green plants is a result of optical excitation.

An excited atomic system may emit photons in two ways: either *spontaneously* or by *induced* emission. In the former case the emission is produced without any external effect (e.g. external force field). The induced emission is triggered by another photon of the same frequency as the emitted one. In photon-induced emission the electron is displaced from a higher-energy level to some lower state. Spontaneous emission occurs statistically in every direction and produces incoherent light. Induced emission, on the other hand, consists of highly coherent photons, which propagate in the same direction as the light-inducing photons. The usual light sources produce only spontaneous emission, and the induced emission is practically negligible. However, lasers are light sources based on induced emission (cf. section 2.6).

2. Absorption. In optical excitation a photon may transfer its energy to an atomic system in three different ways.

(a) The absorbed photon only *perturbs* the electronic state. The perturbation time is approximately the same as the oscillation period of the photon (in the visible range this is approximately 10^{-15} s). The perturbation results in the emission of photons, whose frequency is the same as that of the incident photons. The probability of production of photons with different frequencies is rather low. Light scattering at the same frequency is usually called *coherent* (classical or Rayleigh) scattering, whereas photons with frequencies different from the exciting frequency constitute *Raman scattering* (section 3.3.3). Light of any frequency may produce light scattering, which is also responsible for the phenomenon of light reflection and refraction.

(b) The absorbed photon *excites* the atomic system by raising it to a higher energy level. The life-time of the excited state is generally several orders of magnitude longer than the perturbation time: in the case of allowed optical transitions it is at most 10^{-8} s, but for metastable levels 10^{-3} s or even longer.

The excitation energy may be emitted as a photon, this phenomenon being called *luminescence*. However, the excited atoms or molecules may trigger chemical transformations without any light emission; these processes are *photochemical reactions*. In most cases, however, the excitation energy is transformed into heat (phonon emission), i.e. in a strict sense *light absorption* occurs only if absorbed light energy is converted into heat.

For any given system, excitation is observed only at some well-defined frequency or in a definite frequency range. In the excitation processes, mainly because of scattering and heat conversion, the intensity of the exciting light decreases as it passes through the system (cf. section 2.3.1). The spectral distribution of the light attenuation is characteristic of the absorbing system.

(c) The photon behaves as a classical particle and produces a *photoelectric effect*. With high-energy electromagnetic radiation (X-radiation and γ-radiation) the Compton effect and pair production may also be considerable (cf. section 2.10.2).

2.3. Radiometry—Photometry

In the following sections optical radiation will be dealt with as an energy transporting process and the fact that it may produce a light sensation as well will be considered only in section 2.3.2. Thus first the *measurement of radiation*, i.e. *radiometry*, will be discussed and only subsequently will follow the measurement of visible radiation weighted according to light sensation, i.e. the *measurement of light* or *photometry*. The concepts and relations to be discussed in radiometry can be applied not only in the optical range but all over the complete electromagnetic spectrum.

2.3.1. Radiometry

1. Basic concepts. Let dQ denote the *radiant energy* emitted by a radiation source in time dt. *Radiant flux* is defined as

$$\Phi = \frac{dQ}{dt} \qquad [2.1]$$

i.e. as the energy emitted in unit time. Its unit is W.

The radiant flux emitted by a radiation source in various directions is usually different. The dependence on direction is characterized by the *radiant intensity* defined by the quantity

$$I = \frac{d\Phi}{d\omega} \qquad [2.2]$$

where $d\Phi$ denotes the radiant flux propagated in an element of solid angle $d\omega$ containing the given direction. Thus the radiant intensity gives the flux emitted in unit solid angle[1] in the given direction. Its unit is W sr^{-1}.

In practice it is often necessary to know the radiant flux density at a given place of an irradiated surface, i.e. the energy incident per unit time and surface. This is given by the quantity

$$E = \frac{d\Phi}{dA} \qquad [2.3]$$

where $d\Phi$ denotes the radiant flux incident on the surface element with area dA at the given place. It is called *irradiance*. Its unit is W m^{-2}.

In everyday practice the expression radiant intensity and its symbol I is often used differently from [2.2]. The quantity giving the radiant flux incident on a unit surface perpendicular to the propagation of radiation is also called *radiant intensity*:

$$I = \frac{d\Phi}{dA} \qquad [2.4]$$

where in this case dA is the area of the surface element perpendicular to the radiation. Obviously [2.4] is more closely related to [2.3] than to [2.2]. Its unit too is the same as that of irradiance: W m^{-2}.

[1] "sr" means *steradian*, i.e. the symbol of the unit of solid angle. — In a plane an angle is measured as the quotient of an arc and the radius relating to it. A *solid angle*, on the other hand, is defined as the three-dimensional angular spread at the vertex of a cone, measured by the area intercepted by the cone on a unit sphere whose centre is the vertex of the cone. The total solid angle corresponds to the surface of a sphere of radius r; since this is equivalent to the total space (S), the space is described by the quantity 4π.

The energy radiated by the Sun is 4×10^{26} J s^{-1}, and the intensity at the atmospheric boundary (the Sun–Earth distance is ca. 1.5×10^{11} m) is approximately 1.35×10^{3} W m^{-2} (this is the solar constant). At most only half of this energy reaches the Earth, the rest being absorbed and scattered by the atmosphere. The human eye is most sensitive to yellowish-green light, and an intensity of approximately 2×10^{-12} W m^{-2} can already be perceived.

2. The law of radiation attenuation. On passing through some medium, radiation loses intensity due to scattering and transformation into some other energy, mainly heat (absorption). As concerns the degree of attenuation, we shall consider only the case of a parallel beam striking some medium normally.

Let the intensity of the radiation striking a layer of the medium of thickness x be denoted by I_0, and the intensity of radiation having passed through this layer by I (Fig. 2.2). The intensity decreases exponentially with the increase of x:

$$I = I_0 e^{-\frac{x}{\delta}} \qquad [2.5]$$

where δ is the layer thickness which decreases the intensity by a factor e (e=2.71..., the base of natural logarithms).

The intensity decrease is frequently characterized by the half-value thickness, i.e. the layer thickness D which decreases the intensity by half. Of course, δ is always larger than D:

$$D = 0.693\delta \qquad [2.6]$$

The meaning of δ is obvious, since with $x=\delta$, $I/I_0=e^{-1}$. If $x=D$, from the definition of D we have $I=I_0/2$, and consequently $e^{D/\delta}=2$. If the logarithms of both sides are taken, [2.6] is obtained.

The quantity $\mu=1/\delta$ is the attenuation or *extinction coefficient*. μ is the reciprocal of that layer thickness which decreases the intensity to $1/e$ of its original value: its unit is consequently m^{-1}, cm^{-1}, etc. The substitution of μ into [2.5] leads to

$$I = I_0 e^{-\mu x} \qquad [2.7]$$

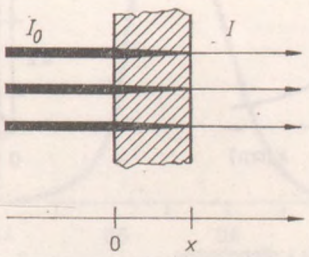

Fig. 2.2. Diagram relating to the Lambert–Bouguer law

while from [2.6] we have

$$\mu = \frac{0.693}{D} \qquad [2.8]$$

From simple mathematical considerations, [2.7] is equivalent to

$$-dI = \mu I\, dx \qquad [2.9]$$

This means that for a sufficiently small layer thickness (dx) the intensity change (dI) is proportional to the layer thickness and the intensity I measured at the point of incidence of the beam on the layer. The negative sign refers to the fact that dI is negative for an intensity decrease.

The law expressed by [2.5] or the equivalent relations [2.7] and [2.9] are known as the *Lambert–Bouguer law*.

The common logarithm (to the base 10) of the quotient I_0/I is the decadic extinction or simply the extinction (often called the optical density), while the natural logarithm (to the base e) of I_0/I is called the natural extinction. For solutions, if μ is proportional to the molar concentration c, the following relation holds: $\mu = \varepsilon c$ *(Beer law)*. The proportionality factor is the *molar extinction coefficient*.

D and consequently δ and μ depend upon the nature of the scattering or/and absorbing medium and also upon the wavelength of radiation.

Figure 2.3 depicts the effect of attenuation for $D = 2$ cm. The decrease in intensity is frequently due to absorption in the narrower sense, if the light scattering is negligible. This explains why the term absorption coefficient is used instead of the extinction coefficient.

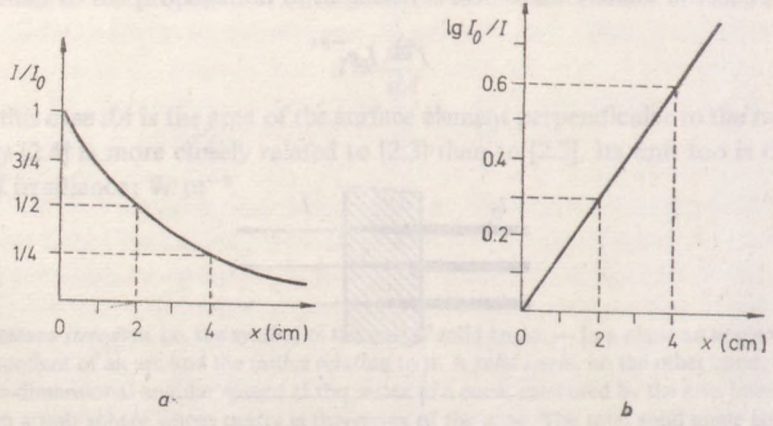

Fig. 2.3. Variation in the relative intensity (I/I_0) and the extinction ($\lg (I_0/I)$) with the layer thickness (x) for $D = 2$ cm

3. Further concepts and quantities

(a) The *reflectivity* (reflectance) of a body is the fraction of the total incident radiation reflected from some site on the surface. The reflectivity of high-quality mirrors (silver surfaces) in the visible range is 0.9–0.96, i.e. 90–96%.

(b) The *transmittivity* (transmittance) is defined by the ratio of the transmitted and the incident energy.

(c) The *absorbance* is the ratio of the absorbed and incident radiation. This quantity is always a fraction. The absorbance of soot for visible light is close to 1, i.e. nearly 100%.

The quantities defined in points (a)–(c) depend upon the angle of incidence. If this is not given, incidence normal to the surface must be assumed. It follows from the definitions that in a given case the sum of the quantities defined in (a)–(c) is 1. If the penetrating energy is considered instead of the incident energy, one obtains the pure transmittance for case (b) and the pure absorbance for case (c). The value of the penetrating energy is obtained by subtracting the reflected energy from the incident energy.

2.3.2. Photometry

1. Spectral luminous efficiency. The sensitivity of the human eye varies with the frequency (or wavelength) of light. Figure 2.4 shows the *average spectral sensitivity*, i.e. the *spectral luminous efficiency* $[V(\lambda)]$ of the human eye. The abscissa represents the wavelength and the ordinate the sensitivity (luminous efficiency). The maximum value of the sensitivity at 555 nm is taken as 1. If a k times higher intensity is required to produce the same sensation of light at some other wavelength, then the sensitivity of the eye for this wavelength is only $1/k$.

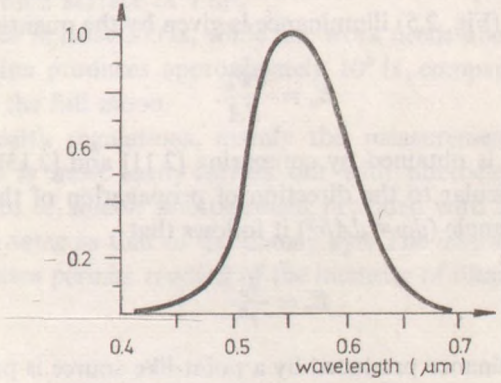

Fig. 2.4. Sensitivity curve of the eye in daylight

2. Photometric quantities. Only the quantities corresponding to the previously discussed radiometric quantities will be discussed here.

(a) *Luminous flux* is a quantity derived from radiant flux by weighting it with the luminous efficiency. It is denoted by Φ_v. The subscript v here and further on refers to visual, i.e. to visibility.

The relation of Φ_v to Φ can be obtained by the following consideration. Let denote $d\Phi$ the radiant flux between the wavelength λ and $\lambda+d\lambda$ and V the luminous efficiency at λ. The luminous flux in the above wavelength range is given by $V\,d\Phi$. The total luminous flux in the visible range can be obtained by integration:

$$\Phi_v \sim \int V\,d\Phi,\ \Phi_v = K\int V\,d\Phi \qquad [2.10a]$$

The proportionality factor K depends on the units chosen. The unit of radiant flux is watt, that of luminous flux is lumen (lm) which will be defined later on. Since according to the definition and the measurements at the maximum of the spectral luminous efficiency ($V=1$) 1 W corresponds to 683 lm, therefore

$$K = 683 \text{ lm W}^{-1} \qquad [2.10b]$$

(b) *Luminous intensity* is related to radiant intensity. In the case of a point-like source in a given direction it is expressed by the quantity

$$I_v = \frac{d\Phi_v}{d\omega} \qquad [2.11]$$

where $d\Phi_v$ denotes the luminous flux propagated in an element of solid angle $d\omega$ containing the given direction. Thus the luminous intensity is the luminous flux propagating in unit solid angle. If the emission of the light source is uniform in every direction, i.e. I_v is the same in every direction, the total luminous flux Φ_o of the light source is obtained according to [2.11] by multiplying I_v by the total solid angle 4π:

$$\Phi_o = 4\pi I_v \qquad [2.12]$$

(c) *Illuminance* corresponds to irradiance. If a surface element of area dA receives a luminous flux of $d\Phi_v$ (Fig. 2.5) illuminance is given by the quantity

$$E_v = \frac{d\Phi_v}{dA} \qquad [2.13]$$

A valuable relation is obtained by comparing [2.11] and [2.13]: $E_v = d\omega I_v/dA$. If the surface is perpendicular to the direction of propagation of the beam, from the definition of the solid angle ($d\omega = dA/r^2$) it follows that

$$E_v = \frac{I_v}{r^2} \qquad [2.14]$$

Consequently the illuminance produced by a point-like source is proportional to the luminous intensity and inversely proportional to the square of the distance. If the

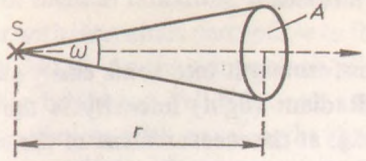

Fig. 2.5. Diagram relating to the definition of light intensity and the intensity of illumination

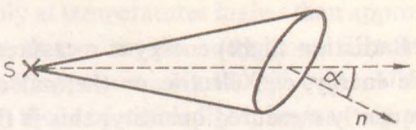

Fig. 2.6. Diagram relating to [2.15]
n: the normal to the surface

surface is oblique with respect to the incident beam (Fig. 2.6), the following relation holds instead of [2.14]

$$E_v = \frac{I_v}{r^2} \cos \alpha \qquad [2.15]$$

which means that the more obliquely a beam strikes a surface the lower the illuminance.

3. Units. In photometry the basic unit is the unit of luminous intensity and the units of other quantities are derived from this (cf. Table 8.1).

The unit of luminous intensity is the candela (denoted by cd). The candela is the luminous intensity, in a given direction of a source that emits monochromatic radiation of frequency 540×10^{12} Hz (i.e. of wavelength 555 nm in vacuum) and has a radiant intensity in that direction of (1/683) W sr^{-1}. This is approximately equivalent to the luminous intensity produced by a simple candle in the horizontal direction. For the production of a given luminous intensity, standard lamps made and calibrated according to internationally accepted standards can be obtained from the various Bureaux of Standards. These lamps play the same role in photometry as the standard metre bar in longimetry or the standard resistances in electrical measurements.

The unit of luminous flux is the lumen (denoted by lm) which is the luminous flux emitted by a light source of 1 cd in unit solid angle.

The unit of illuminance is the lux (denoted by lx). 1 lm luminous flux produces 1 lux illuminance on a surface of 1 m^2.

Reading requires at least 200 lx, while fine work needs about 1000 lx. At noon in summer the sunshine produces approximately 10^5 lx, compared with approximately a few tenths lx by the full moon.

As concerns health regulations, mainly the measurement of the illuminance is important; this is most easily carried out with photoelements. The sensitivity curve of a selenium or silicon photoelement provided with an appropriate filter is approximately the same as that of the human eye. The instrument connected to the element in most cases permits reading of the intensity of illumination directly in lux units.

2.3.3. Measuring methods

Radiation (light) energy is measured by transforming it into some easily measurable energy, e.g. electric or thermal energy. Radiant (light) intensity is the most frequently measured quantity; this is the case e.g. at the measurement of the extinction coefficient or the spectral distribution of the intensity in the emission spectrum of a radiation source.

Intensity measurement based on the *photoelectric effect* is a readily available, very sensitive and subsequently frequently used method, since the photocurrent is directly proportional to the intensity incident on the photoelectric cell. In the visible and ultraviolet range photocells with Cs, Na, K, etc. cathodes are used together with Si, GaAs, etc. photodiodes while those in the infrared range have lead sulphide, lead selenide, etc. photoconductors. One drawback of these methods is the dependence of the photoelectric effect on the radiation (light) frequency. As a consequence, the photoelectric methods are used only to measure the intensities of radiation (light) of identical spectral distributions. If different frequencies are compared, the frequency-dependence of the photocell or photoelement, etc. must be taken into consideration.

If a *thermocouple* or thermopile is used, the incident radiation (light) strikes the soldering seam, which is coated with some appropriate absorbing material (e.g. for visible light soot). The seam is heated up by the absorbed radiation, and the thermocurrent produced is proportional to the intensity of the incident radiation. This method is readily applicable throughout the whole spectral range. Its great advantage is that it is independent of the frequency. – Detectors based on the *pyroelectric effect* are similarly advantageous; they are more sensitive than the above-mentioned ones but respond only to the change of radiation intensity.

For the measurement of radiation the *photographic effect* is often used. At low energy the darkening of the light-sensitive layer is proportional to the incident energy. Accordingly, not the intensities, but the products of the intensities and exposure times are compared in this case. With equal exposure times the darkening is proportional to the incident intensity. Because of the frequency-dependence of the darkening, the considerations discussed in connection with the photoelectric effect hold here too. A further disadvantage is the different photosensitivities of different plates, and even of the various sites on a given plate.

2.4. Thermal radiation

Radiation produced at the expense of thermal energy (phonon space) of a body, and depending only on the temperature of this body, is thermal radiation. Every body emits thermal radiation at any temperature. As long as the temperature of the body is lower than approximately 750 K, it emits practically only infrared light, and consequently its radiation cannot be observed by the human eye. The visible compo-

nents of thermal radiation, with wavelengths shorter than those in the infrared range, appear with intensities perceptible to the eye only at temperatures higher than approximately 750 K. As the temperature rises above this value, the body glows first dark red, then bright red, yellowish-red, and finally at approximately 1800 K white. Ultraviolet radiation, with wavelengths shorter than those of visible light, begins only at a temperature higher than approximately 2000 K with such intensity that its biological effects should be taken into account.

More exact data are given in Fig. 2.7, which illustrates the wavelength (λ) dependence of the emittance for various temperatures T of an absolute black body.[2] An incandescent lamp, a heating radiator, the human body and also the Sun radiate almost as black bodies.

In the case of the absolute black body the emission in the wavelength range between λ and $\lambda+d\lambda$ is characterized by the quantity $E(\lambda, T)\,d\lambda$, where $E(\lambda, T)$ denotes the radiant flux emerging perpendicularly to the emitting surface in unit wavelength range, surface area and solid angle. $E(\lambda, T)$ is the emittance of the absolute black body. With the aid of $E(\lambda, T)$ the emittance of any other body, $e(\lambda, T)$ can be determined knowing its absorbance $a(\lambda, T)$, since according to *Kirchhoff's law* the following equation holds:

$$e(\lambda,T) = E(\lambda,T)\,a(\lambda,T), \text{ i.e. } E(\lambda,T) = \frac{e(\lambda,T)}{a(\lambda,T)} \qquad [2.16]$$

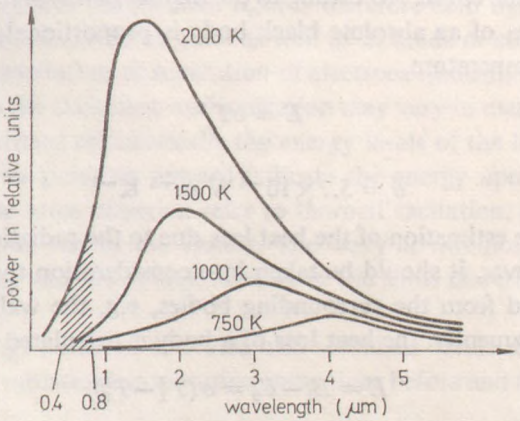

Fig. 2.7. Spectral energy distribution in black body radiation
The visible region is shaded

[2] The term *absolute black body* refers to a body whose absorbance is 1, and transmittance zero at all temperatures and all wavelengths. The absolute black body is an ideal limiting case, but it can be attained to a good approximation. Let us take, for example, a closed metal box with a sooted inner surface and a small hole in one of its faces. The light incident through this hole becomes continually weakened by multiple reflection, and in the case of a properly made box only a very small amount of the incident radiation will emerge through the hole. Thus, the hole behaves as a black spot on the surface of the hollow body. If this body is heated to different temperatures, the hole radiates virtually as an ideal black body at the respective temperatures.

[2.16] expresses the experimental fact that at a given T and λ the quotient of $e(\lambda, T)$ and $a(\lambda, T)$ is the same for every body. If a body emits some radiation more strongly, it also absorbs the same radiation more strongly, and conversely. In general, $a(\lambda, T) < 1$, and therefore $e(\lambda, T) < E(\lambda, T)$; this means that the emissivity of any body at a given wavelength is smaller than that of an absolute black body at the same temperature.

Figure 2.7 demonstrates that with increasing temperature the maxima in the curves are shifted towards shorter wavelengths. The energy distribution in the spectrum of the Sun is approximately the same as that of a black body at 6000 K. At this temperature the maximum in the distribution curve (not shown in the Figure) is already in the visible range. Thus, the human eye is sensitive in the range of the maximum of solar radiation. Besides ultraviolet radiation, solar emission contains a considerable amount of X-radiation. However, X-radiation and the very short-wavelength ultraviolet radiation are absorbed by the atmosphere (especially by the ozone in the upper atmosphere) so that radiation with a wavelength shorter than 290 nm does not reach the Earth's surface. Of course, infrared radiation of very long wavelength is also found in the spectrum of the Sun; this is detectable as a group of ultrashort radiowaves.

Figure 2.7 also shows that with increasing temperature the emitted power increases at every wavelength. The *emitted total power at a given temperature* is demonstrated by the area under the curve. According to the *Stefan–Boltzmann law* the emitted total power per unit area of an absolute black body is proportional to the fourth power of the absolute temperature

$$E = \sigma T^4 \qquad [2.17a]$$

where

$$\sigma = 5.7 \times 10^{-8}\ \mathrm{W\ m^{-2}\ K^{-4}} \qquad [2.17b]$$

[2.17] allows the estimation of the heat loss due to the radiation of a body at temperature T_1. However, it should be taken into consideration that the body also absorbs heat radiated from the surrounding bodies, e.g. the walls, at a temperature T_2 ($T_1 > T_2$). Consequently, the heat loss of a body is calculated from

$$E = E_1 - E_2 = \sigma(T_1^4 - T_2^4) \qquad [2.18]$$

At rest, the human organism releases somewhat more than 50% of its heat in the form of radiation from the surface of its body into its environment at room temperature. From the aspect of radiation, at 303 K the human body behaves as a body with an absorbance of 95%. According to [2.18] the emitted power of a living organism in an environmental temperature of 293 K is approximately 60 $\mathrm{W\ m^{-2}}$. The emitted energy falls in the infrared range of the spectrum, with a maximum at 9.5 μm.

[2.18] permits the estimation of T_1 (if T_2 is known) by measuring E, or determination of the difference $T_1 - T_2$. As an example, this possibility is used for diagnostic purposes when the temperature of the skin surface is measured at different points by means of thermal radiation (infradiagnostics; cf. section 5.6.5).

2.5. Luminescence

Light emission which cannot be attributed to the phonon space, but is a result of some other excitation, is called *luminescence*. Luminescent light may be excited by electromagnetic radiation, corpuscular radiation, the effect of an electric field, or chemical processes. In the following we shall mainly deal with the luminescence produced by light, though the conclusions can be extended to other types of excitation. By the above definition, light scattering produced by light might be regarded as luminescence, but it is a very fast process, occurring in less than 10^{-10} s. However light emission is regarded as luminescence only if it follows excitation on the average after longer than 10^{-10} s. Thus, the definition given should be complemented by excluding light scattering and other fast light-emitting processes, such as Cherenkov radiation from the notion of luminescence.

In luminescence the simultaneously excited atoms and molecules (in short the luminescence centres) do not emit simultaneously. When the excitation ends, the excited centres do not stop emitting, but decay during some shorter or longer time. This decay time is possibly only 10^{-6} s, but it may be several hours or even days.

The intensity of the luminescent light (in a given wavelength range) is always higher than the intensity of the thermal radiation of the emitting body at the given (usually room) temperature; luminescent light is therefore cold light.

Luminescence can be observed in gases as well as in fluids or solids. The primary process is always the excitation or separation of electrons (ionization). However, the process subsequent to the excitation and ionization may vary in many ways.

Figure 2.8 demonstrates schematically the energy levels of the luminescent molecules. The thick arrows pointing upward indicate the energy uptake at excitation. The thin arrows in the same direction refer to thermal excitation, an energy uptake resulting from a process within the system. The wavy arrows pointing downwards indicate light emission, and the straight arrows in the same direction correspond to thermal energy losses.

The left side of Fig. 2.8*a* demonstrates also vibration levels related to electron states. This illustrates radiationless vibration transitions before and after the emission.

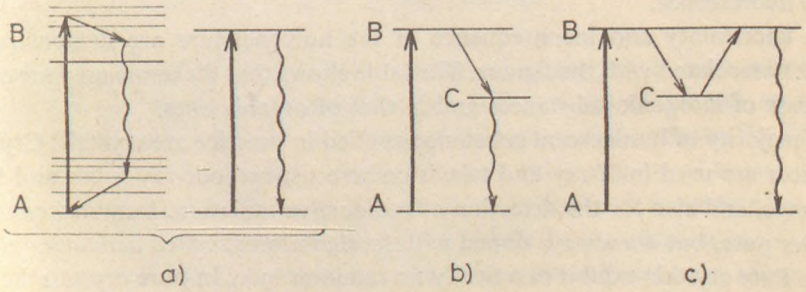

Fig. 2.8. Examples for mechanism of luminescence

These types of transition are rather universal, which explains in itself the experimental fact that the energy of the emitted photons is generally not larger than that of the absorbed ones *(Stokes' rule)*. The right side of Fig. 2.8*a* illustrates the same process, however, for simplicity, the vibration levels are not shown and the ground as well as the excited states are represented by single lines. The same simplification has been applied also in the other two figures.

In Fig. 2.8*b–c* beside the *B* excitation level there is another one, denoted by *C*. This is a *metastable* level, which means that in the $C \rightarrow A$ transition emission occurs only with a small probability (cf. section 1.2.2). Figure 2.8*b* illustrates the case, when the excited molecule first gets from level *B* to level *C* by a radiationless transition, and from there with a delayed emission back to the ground state.

In the process demonstrated by Fig. 2.8*c* the electron in the metastable state first attains level *B* by the aid of thermal energy, and from there returns to the ground level. Figures *b* and *c* demonstrate also the processes in the crystal-phosphors (see below) if *A* and *B* represent the valence as well as the conduction band, and *C* represents the donor or acceptor levels (cf. 1.4.6).

In connection with the phenomena of luminescence the terms *fluorescence* and *phosphorescence* are frequently used. Formerly, when the mechanism of luminescence was not sufficiently known, the classification was made according to the decay time. The luminescence was called fluorescence if with the cessation of the excitation the luminescence too practically ended, while phosphorescence referred to a longer afterglow. More recently — especially for inorganic substances — the classification depends upon the temperature dependence of emission. The luminescence is called fluorescence if a temperature range exists in which the emission is practically independent of temperature. If the emission is not temperature independent the emitting transition is referred to as phosphorescence.

Figure 2.8*c* demonstrates a case when temperature plays an important role in the process of elevation from level *C* to *B*, since at higher temperature the system possesses the energy necessary for elevation in a given time with a higher probability than at lower temperature. Consequently with increasing temperature the emission associated with the *B–A* transition will also become faster and more intensive. Considering the temperature dependence Fig. 2.8*c* illustrates phosphorescence, while Figs *a* and *b* refer to fluorescence.

The uncertainty and inconsequence of the nomenclature are demonstrated by Table 2.1 associated with the figures. The table shows that the terms used are different in the case of inorganic substances and in that of organic ones.

The majority of luminescent substance applied in practice are crystals. Crystalline phosphors are used in X-ray and television screens, cathode-ray tubes and fluorescent lamps, and also for the detection of radioactive radiation. Luminescent crystals are never pure, but are always doped with foreign atoms, called *activators* or *photocentres*. Pure crystals exhibit practically no luminescence. In pure crystals the excitation energy is transformed into phonons. The colour of the emitted light depends both

Table 2.1

Terms used for Fig. 2.8

Substance	Fig. 2.8		
	a	*b*	*c*
Inorganic	fluorescence	fluorescence	phosphorescence
Organic	fluorescence	phosphorescence	delayed phosphorescence

on the basic material and on the incorporated activator. By a suitable choice of the activator, practically any colour can be selected even at the same basic material and luminescent phosphors emitting any desired wavelength can be made. For instance, the colours of fluorescent lamps can be designed to accord to the requirements of industrial health regulations.

As mentioned previously, the introductory act of luminescence is the excitation or ionization of atoms or molecules. Various types of luminescence exist, depending upon the process of producing the required exciting and ionizing energy. *Photoluminescence* is luminescence produced by light, while *radioluminescence* is due to ionizing radiation. The luminescence produced by fast electrons is *cathodoluminescence*. The energy released by certain chemical processes may also result in luminescence; this is *chemoluminescence*, which produces the luminescence of some animals or the light of phosphor due to slow oxidation in the air. The name phosphorescence is derived from this process. The fracture of friction of certain materials may also result in luminescence *(triboluminescence)*. For instance, when a lump of sugar is broken into two parts, a weak gleam may be observed in the dark. Luminescence may similarly result from an electric field *(electroluminescence)*.

Luminescence is a rather complex phenomenon, and not all its details are understood. Its study is not only of practical importance but also plays a significant role in structural research, especially in the case of biological macromolecules (cf. section 3.3.1).

2.6. Light sources

In some light sources the photon emission of substances at high temperature is produced at the expense of thermal energy. These types of sources emit thermal radiation (cf. section 2.4). The most important natural light source, the Sun, and a large number of artificial light sources belong in this category. Common incandescent lamps with tungsten filaments glow at about 2700 K: their spectrum is approximately the same as the emission spectrum of black body radiation at this temperature (cf. section 2.4). The glass bulb, however, transmits only the range of the spectrum between 350 nm and 2.8 μm. In special cases, if just the emission of infrared light is

desired, tungsten spiral lamps, called infralamps, glowing at a lower temperature of approximately 1300 K, are used. The *sollux lamp* is a high-power tungsten spiral lamp glowing at roughly 3300 K. These lamps are provided with filters to eliminate the long-wavelength infrared radiation from the spectrum. The near-infrared radiation of these lamps is used in therapy.

As concerns *luminescent* light sources, primarily *metal vapour* lamps are of interest. In these lamps, metal (e.g. mercury, sodium) vapour is contained in a glass or silica tube. Electrodes protrude into the sealed tube, and the vapour is excited by an electric discharge produced by the voltage across the electrodes. These types of lamps yield line spectra. The 589 nm spectral line of the *sodium lamp* (the sodium D line) is used in laboratory practice as monochromatic light. Of the metal vapour lamps, mercury lamps are of special medical interest. The tube walls of these lamps are made of silica, which transmits ultraviolet light (down to 200 nm). For this reason they are frequently referred to as quartz lamps. Two types are distinguished: low (1–100 Pa) and high (0.01–10 MPa) pressure mercury vapour lamps. *Germicidal* lamps are of the first type. Approximately 75% of the emitted energy of these lamps is observed as the 254 nm spectral line. High-pressure lamps give a spectrum of many broad lines in the ultraviolet range. (After high-pressure lamps are switched on, the intensity of the emitted light increases for some time and becomes stable only after a few minutes.)

Because of their economicalness, *fluorescent lamps* (or *F-tubes*) are gaining ground in illumination technics. In most cases, these tubes are low-pressure mercury vapour lamps, whose inner walls are coated with some luminescent material. 254 nm light excites the luminescent substance, which emits visible radiation (optical excitation). The tube wall is made of glass which transmits only the visible light. By variation of the luminescent material, the composition of the emitted radiation, i.e. the colour of the light, can be changed. *Erythemal lamps* operate on a similar principle. In these lamps the bulk of the emitted light lies in the biologically most effective range, 280–320 nm. The lamp walls are made of a special glass (uviol) which, while absorbing, at 254 nm, transmits the biologically favourable ultraviolet radiation. More recently *xenon lamps* made of silica glass have been applied in phototherapy. These lamps supply a spectrum very close to that of the Sun, and are thus very suitable for obtaining nearly the same effects as the Sun.

In the past few years a basically new light source has been developed, the *laser* (abbreviation for light amplification by stimulated emission of radiation). In contrast with the commonly used light sources lasers yield an extremely *coherent, monochromatic* light beam, which consequently *can be well collimated and focused onto a very small surface.*

Monochromatism or coherence may be understood from the following reasoning.

The width of spectral lines is always finite. Consequently, spectral lines are characterized by frequency intervals rather than by a single frequency value. It follows that a single photon cannot be thought of as a single infinite harmonic wave (sine curve) extending in both space and time. Instead, we are dealing with a *group* of harmonic waves. The group covers several frequencies (wavelengths)

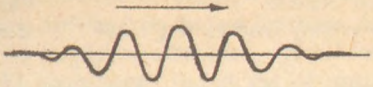

Fig. 2.9. Wave train of finite length

distributed in a finite range. The resultant of this group of harmonic waves is not infinite, either in time, or in space. It is a *wave train* of finite length (Fig. 2.9), and the resulting amplitude is everywhere zero except along this finite length. Light may be visualized by these *wave trains* or *wave packets of finite length*. The length of the wave packet is the *coherence length*; it is small if the frequency interval, also called the wave band, is large, and in turn a large coherence length is accounted for by a narrow wave band. With normal light sources the coherence length extends from a few nm to at most a few cm. Lasers, on the other hand, produce highly monochromatic light with a considerably larger coherence length (10^2–10^8 m).

The usual light sources emit individual wave packets at different times and with different phases. Further, the planes of the oscillations are also distributed statistically. Light interference can be produced if different parts of the same wave train are brought to meet. Expressed in a different way, interference is observed if the path difference between the light waves is smaller than the coherence length. In the case of usual light, because of the small coherence length, interference is obtained only under special experimental conditions. However, laser light may produce interference patterns even of simple objects of everyday life by means of *holography* (see below).

Lasers are made of various materials; for instance, artificial ruby crystals (Al_2O_3 doped with Cr^{3+} ions) a few cm long and with a diameter of 1–2 cm are frequently used. Both continuously operating lasers and pulse lasers are made. The former are mainly gas lasers, while the latter are produced from solids or liquids. Lasers of semiconducting materials may operate both continuously and in pulse mode. A given laser material radiates only at a certain wavelength (or wavelengths). However, the choice available is so large that practically any demand can be satisfied, from the UV to the far IR range.

In order to understand the operation of lasers, consider Figs 2.10. and 2.11. The laser effect is based on induced emission (cf. section 2.2). In the case of gases their atoms or molecules participate in the process while with solid-state lasers the dopants (e.g. Cr^{3+} ions in ruby lasers) take part. Energy is first communicated to the laser material by exciting a large proportion of the molecules. This is depicted in Fig. 2.10 by the arrow pointing from E_0 to the broadened energy level E_1. In most

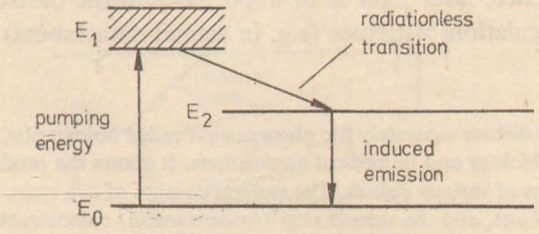

Fig. 2.10. Three-level laser system
E_0 denotes the ground level; E_1 and E_2 are the excited levels

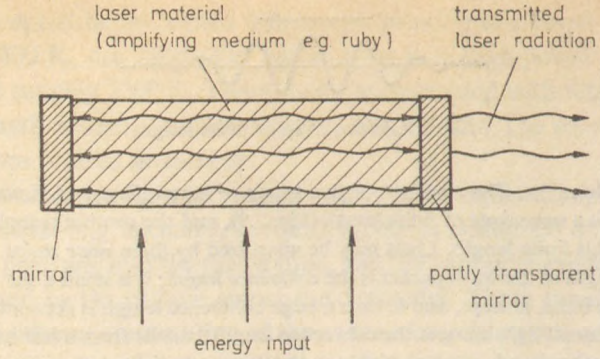

Fig. 2.11. Outline of a laser set-up
The horizontal lines between the mirrors denote the oscillating laser light

cases this energy pumping is achieved with light (e.g. flashlight). The excited molecules do not return from the E_1 level to the ground state, but with great probability will occupy the level E_2. The transition $E_1 \rightarrow E_2$ is radiationless. Since the level E_2 is metastable, the molecules return to their ground state only with low probability; however, there always exists the possibility of an induced emission. This is started by spontaneously emitted photons, which are reflected from mirrors, thereby generating further photons. The process is repeated several times within a fraction of a second (Fig. 2.11). In this way a considerable proportion of the energy stored in the laser material emerges through the semitransparent mirror, which operates in two directions by allowing both the reflection and the emergence of the laser light. The laser may be regarded as a feedback amplifier operating in the optical range.

With lasers in pulse operation a very large power density (intensity) can be obtained on a small surface, so that every presently known substance evaporates. For instance, a single pulse of a ruby laser operating at 694.3 nm yields an energy of 10 J within one ms. If this energy is focused on an area 10 μm in diameter, a power density of 10^{14} W m^{-2} may be attained. This is approximately 10^{12} times larger than the maximum power density of the Sun on the surface of the Earth. Material evaporation is used in machining, e.g. for drilling holes or milling. Large-scale experiments are currently being carried out to investigate the effects in more detail, and to establish the possibilities of the practical application of laser light. The investigations have also been extended to the role of the considerable coherence and its effects.

In medical practice, laser light is of importance in the destruction of small size tissues, in the coagulation of tissues (e.g. in retinal detachment) and in the healing of ulcers.

It is worthwhile to discuss separately the phenomenon called holography, which appears to be a promising method in biology and in medical applications. It allows the production of three-dimensional enlarged pictures of various objects. The method consists of two steps. The first step *produces the hologram* of the object, and the second step *(reconstruction)* reconstructs the picture from the hologram. Every hologram is an *interference pattern* on a photographic plate or film (Fig. 2.12). The light from the light source strikes the plate or film via two routes: after reflection from the

object *(object wave)*, and after reflection from the mirror *(reference wave)*. The hologram is produced by the interference of the light waves arriving from the two directions. A system of interference fringes of various densities is produced on the photographic plate, which does not seem to yield any information about the object (Fig. 2.13, Supplement). In fact, the information content is more than that given by a simple photograph since the amplitude and the phase of the waves reflected from the object play equally important roles in the formation of the interference pattern. Thus, the hologram contains the full information collected by the reflected light from the object. The usual photographic picture provides information only about the amplitude of the reflected beam: the darkening of the light-sensitive material is proportional to the square of the amplitude. In the second step of holography (Fig. 2.14) the hologram is transilluminated, which results in two images of the object, one *real* and the other *virtual*.

The production of a hologram requires light capable of interference over a long path, which means that the light should be a laser. The reconstruction does not necessarily demand the use of a laser, though a really good-quality image cannot be obtained without coherent light. The wavelength in the reconstruction need not be the same as that used in making the hologram.

The main properties of the holographic method and images may be summarized as follows.

(a) No lenses are required, either to make the hologram or for the reconstruction.

(b) The reconstruction is three-dimensional. If at inspecting the image the head is moved in the proper direction and to a proper extent, previously concealed details become visible.

(c) The image of the whole object can even be reconstructed from merely a part of the hologram with loss of only some of the finer details. The more details are lost, the smaller the domain of the hologram used to produce the picture. Dust, a faulty spot in the emulsion, or any other fault, usually covers only a small section of the hologram, and such imperfections do not seriously interfere with the quality of the reconstructions.

(d) Several holograms can be made on the same photographic plate. For this purpose, only the angle of incidence of the reference beam must be altered each time a new hologram is made. In reconstruction the photographic plate is to be viewed from different angles; this can easily be achieved by the rotation of the plate.

In the reconstruction, *magnification* is obtained in two different ways. In one method the wavelength of the reconstructing light is longer than that used to produce the hologram. Thus, the hologram may be made with ultraviolet radiation for instance, and subsequently reconstructed with yellowish-red light. The magnification in this case is given by the ratio of the two wavelengths. Another method, similarly simple, puts the hologram in the path of a divergent beam. In the case outlined in Fig. 2.15 the magnification is given by $(a+b)/a$. Combination of these two methods leads to magnifications of several hundredfold. The magnification can be further increased if the reconstructed

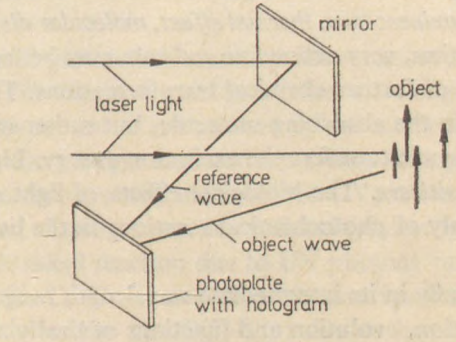

Fig. 2.12. **Production of a hologram**

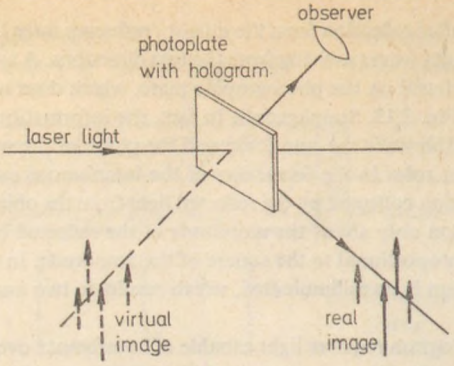

Fig. 2.14. Image reconstruction from a hologram

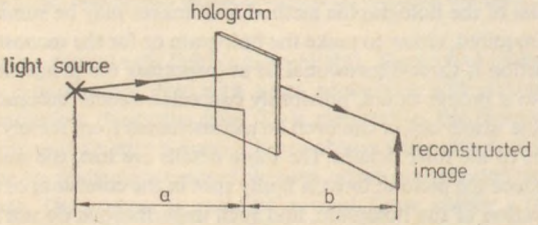

Fig. 2.15. Magnification with the application of a divergent beam

image or its details are investigated microscopically. The resolution of the image is determined by the wavelength used to produce the hologram. The holographic method is particularly advantageous when moving objects difficult to follow with the normal traditional microscope are to be observed.

2.7. The effects of light

The primary effect of light in every case is *excitation,* and with photons of sufficiently high energy it is *ionization.* The secondary phenomena, which are consequences of the primary effect, may differ even for a given substance and wavelength. *Examples* of such phenomena are *luminescence, thermal effect, molecular dissociation,* and so on. In the course of dissociation, very active free radicals may be formed, which in turn may become the sources of further chemical transformations. The dissociation may possibly take place not in the absorbing molecule, but rather in some other one to which the absorbing molecule transfers the excitation energy. Light-absorbing molecules operate as *photosensitizers.* The biological effects of light are consequences of the above processes mainly of photochemical reactions in the living organism.

1. Visible light (especially in its intermediate wavelength range) is of fundamental importance in the formation, evolution and functions of the living organism. *No life could exist on the Earth without light.* The most essential biological effect of visible

light is *photosynthesis*, the basic life process of green plants. Plants synthetize organic compounds from inorganic ones by means of light absorption, while gaseous oxygen is released. The primary process is the excitation of the chlorophyll molecules of the plants. If the chlorophyll bound to proteins transfers the absorbed light energy to water molecules, the latter may dissociate into H and OH radicals *(photolysis)*. These free radicals formed from water react with various compounds to produce oxygen and also products which, by binding and transforming atmospheric carbon dioxide, finally yield carbohydrates. The essence of the process of photolysis is the formation of carbohydrates from carbon dioxide (and water). In this way the absorbed light energy is accumulated in the form of chemical energy in the assimilating organisms.

The chemical energy produced from light energy supplies a predominant proportion of the stored energy of the *whole living world*. The development of plants permitted the development of animal and other organisms which are not photosynthetizing and whose formation and preservation depend on the opposite process, viz. breathing and combustion. The oxygen necessary for these functions has been and still is being formed in the atmosphere of the Earth as a result of photosynthesis. Consequently, the plant organic materials produced by photosynthesis supply the energy necessary for preservation of the life of animal and other organisms, which synthetize their own organic compounds by transforming these materials. Thus, from the viewpoint of energy the living world can be divided into two parts, the energy-storing and the energy-consuming groups. These processes are in biological equilibrium.

The secondary light effects include further biological effects. The most important of these enable the living organism to obtain information about its surroundings. This has led to the formation of special sense organs in higher-order animals.

2. The ultraviolet (UV) range may be divided into three parts from a biological aspect:

UV *A* range:	315–400 nm
UV *B* (Dorno) range:	280–315 nm
UV *C* range:	below 280 nm

The *Dorno range* is particularly significant as concerns biological efficiency. Under the conditions on the Earth, the effect of the wavelength range below 180 nm is negligible, since this radiation is absorbed by the atmosphere.

UV light covers the energy range corresponding to a photon energy of approximately 3–7 eV, and consequently the absorbed UV photon energy is sufficient to split chemical bonds and thereby promote chemical (photochemical) processes. Thus, an important photochemical reaction due to UV photons results in dimerization of the pyrimidine bases (T, C, U) in nucleic acids and systems containing nucleic acids (viruses, cells). In this case the C(5)–C(6) double bonds of two pyrimidine rings stacked over each other in the polynucleotide chain (see Fig. 1.31) are split, and a

cyclobutane ring (dimer) is formed from the C(5) and C(6) atoms of two pyrimidine rings.

All biological effects of UV light can be ascribed to photochemical phenomena, though the actual processes are not always clearly understood. Figure 2.16 illustrates some important biological effects of UV light or rather the spectral distribution of these effects. The abscissa represents the wavelength, and the ordinate the effectiveness, 100 being taken as the maximum effect. These types of curves are the *action spectra.* Comments are made only with reference to curves *A* and *D*. The maximum in curve *D* lies at about 260–270 nm, which corresponds to the absorption maximum of DNA (cf. Fig. 1.36). This allows the conclusion that the bactericidal effect may be a consequence of photochemical reactions in the bacterial chromosomes. The antirachitogenic action spectrum (curve *A*) is interpreted similarly, since it shows a striking resemblance to the absorption spectrum of the provitamin of vitamin D.

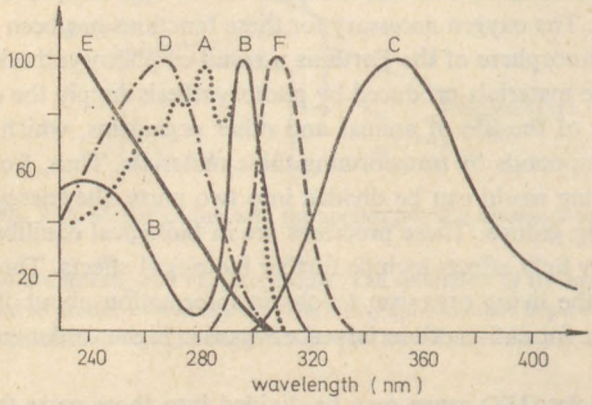

Fig. 2.16. Spectral distribution of the main biological effects of ultraviolet radiation
A: antirachitogenic effect; *B:* erythema; *C:* pigmentation; *D:* bactericidal effect; *E:* conjunctivitis; *F:* carcinogenic effect

3. Infrared (IR) light with a wavelength above 760 nm (the photon energy is less than 1.5 eV), exerts biological effects only by transforming the light absorbed by the tissues into heat. In this respect, mainly the near infrared range is effective, this radiation penetrating up to a distance of 20 mm. The bactericidal, antiphlogistic and analgetic effects are related to the thermal effect. The details of the mechanism of action of IR light have not been elucidated.

2.8. On X-rays in general

X-radiation is produced whenever electrons become stopped[3] after striking some target with a sufficiently high velocity.

[3] X-radiation is also induced by other charged particles, but for practical purposes only electrons are used.

The practically important effects may be summarized as follows:

excitation of luminescence: certain materials (e.g. barium platinocyanide, calcium tungstate, zinc silicate, zinc sulphide doped with silver or copper) luminesce in response to X-ray irradiation;

photographic effect: a photographic plate is darkened similarly as in the case of light;

ionizing effect: the electrical conductivity of some materials is increased (this phenomenon is especially well observable with gases);

chemical effect: in water, for instance, hydrogen peroxide is produced;

biological effect: e.g. the production of morphological and functional changes in cells.

The primary effect produced by X-rays in atoms is in every case *excitation* or *ionization.* All the other effects are only consequences (secondary, tertiary effects and so on), i.e. indirect effects. Particularly complex processes precede the biological effects. The primary phenomena excite chemical processes in the molecules making up the cell; the biological effects are a result of these processes.

The common character of all these effects is the transformation of the X-radiation energy into some other energy. Beside these effects, the *formation of secondary X-radiation (X-ray scattering)* is of importance. This is a fundamental effect too, and accompanies *always* the propagation of X-rays in some medium.

The *detection* and *measurement* of X-rays are generally carried out via one of the effects listed. For example, in diagnostic X-ray irradiation the physician obtains a shadow picture on a luminescent screen or a photographic plate, and generally measures the radiation incident on the body, or absorbed by the body, by means of the ionization of the air or the darkening of the photographic plate due to the radiation (cf. section 2.15).

The wavelength of the X-radiation used in medical practice lies in the range 5–120 pm. This corresponds to a photon energy of 0.2–0.01 MeV. However, in recent decades X-rays of shorter wavelength (less than 1 pm), and consequently of higher photon energy (up to several MeV), have also become of increasing importance.

Though generally not valid, it is accepted in medical practice that the penetrating power of X-rays of shorter wavelength is greater than that of X-rays of longer wavelength (cf. section 2.10). The shorter wavelength radiation with its higher penetrating power is said to be *hard*, whereas the radiation of lower penetrating power is *soft*.

X-rays are used for both diagnostic and therapeutic purposes.

The *diagnostic* application is based upon the fact that the various tissues absorb X-radiation to various degrees. The soft tissues are more transparent to X-rays than, for instance, the bones. Consequently, if the organism is transilluminated, brighter and darker domains are observed, depending upon the absorbance of the tissues.

In radiation effects, only the quantity of absorbed radiation is important in fact. The different cells show various sensitivities, young and actively dividing cells being especially sensitive. For this reason, every tissue and organ in which intensive cell regeneration is taking place, such as the bone marrow, the lower skin layers, the gonads, etc., should be protected with special care. For the same reason, certain pathologically reproducing cells are destroyed more quickly. This destructive effect is in part the basis of the *therapeutic application* of X-radiation (e.g. in the treatment of malignant tumours).

2.9. X-ray sources and their spectra

1. X-ray tube. In medical practice highly evacuated sealed tubes made of glass are generally used to produce X-radiation. The construction is outlined in Fig. 2.17. The electrons are supplied by the hot cathode, opposite which the anode (anticathode) is placed. A voltage (usually 10–400 kV) between the cathode and anode accelerates the electron current, and the X-rays are produced by the electron impact on the anode. Only a few tenths of a percent of the energy of the electrons is transformed into X-radiation, the rest being converted into heat and raising the temperature of the anode.

The spot on the anode where the X-rays are produced is the *focus* of the tube. The smaller (the more point-like) the focus, the more perfect the shadow images obtained on the luminescent screen or the photographic plate. In therapeutic tubes the area of the focus is less important whereas the performance of diagnostic tubes is much better if the focus is more point-like. The focus will be small if the electron beam striking the anode is concentrated; this can be achieved by applying a suitable electric field (electric lens). The X-radiation leaves the focus with maximum intensity nearly normal to the direction of the electron beam. In practice these rays are used; the other parts of the tube are covered with a lead coating, which absorbs the radiation propagating in other directions.

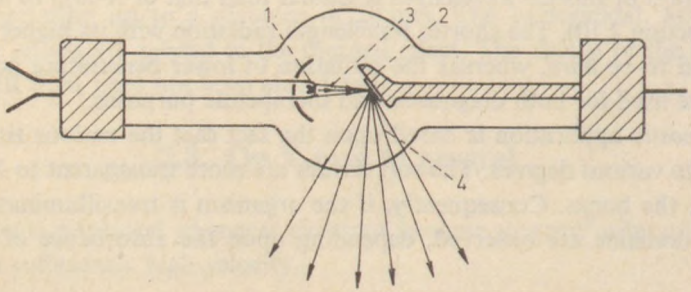

Fig. 2.17. Outline of an X-ray tube
1: hot cathode; *2:* anode (anticathode); *3:* cathode rays; *4:* X-rays

2. Particle accelerators. With X-ray tubes, depending upon the voltage across the tube, the maximum energy of the electron beam is only a few tenths of a MeV. In order to produce higher-energy electrons, and consequently harder X-rays, particle-accelerating equipments, recently mainly linear accelerators, developed in nuclear physics are applied (cf. section 2.14.7).

In medical practice both electrons with high energy (up to ca. 50 MeV) and X-ray induced by these electrons are used.

3. Bremsstrahlung and characteristic radiation. An X-ray tube simultaneously emits waves of various wavelengths and moreover the emitted power is wavelength-dependent. The curves in Fig. 2.18 show the power emitted in the various wavelength ranges. The kV values relating to the curves are the voltages on the tube (the accelerating voltages of the electrons). The following characteristic properties are observed.

(a) The tube radiates in a broad wavelength range. Every wavelength is represented within this range, i.e. *the spectrum is continuous.*

(b) The left side of the spectrum has a sharp cut-off, which shifts towards shorter wavelengths as the voltage is increased. There is no limit on the right side, the emitted power gradually decreasing with increasing wavelength.

(c) Disregarding for the present the peaks shown in Fig. 2.18*b*, only one, well-developed maximum is observed; this points to the existence of a distinct wavelength represented by the maximum energy in the continuous spectrum. With increasing voltage, this maximum shifts towards shorter wavelengths. Neither the short wavelength cut-off nor the position of the maximum depends on the material of the anode.

(d) If the voltage across the electrodes in the tube is sufficiently large, sharp peaks appear at certain sites in the curve (Fig. 2.18*b*) indicating that some wavelengths are present in the spectrum with high power. This leads to the conclusion that the spectrum of an X-ray tube is actually composed of two parts. At lower voltage only the above continuous spectrum appears (Fig. 2.18*a*), but with increasing voltage a *line spectrum* is superimposed on the continuous one (Fig. 2.18*b*).

The two types of X-ray spectrum are due to two kinds of origin of radiation. *Bremsstrahlung* gives the continuous spectrum, whereas the other type, the *characteristic radiation,* is responsible for the line spectrum. As indicated by its name the Bremsstrahlung is produced by electrons colliding with the atoms and subsequently being stopped by the atomic force field (or more precisely by the atomic nucleus rather than the whole atom). The characteristic radiation, on the other hand, is produced by the atoms of the anode, and is characteristic of the anodic target material. In this case too the radiation is initiated by electron impact: however, in this case the X-radiation is due to the atoms excited by the electrons and not to the electrons themselves (cf. section 2.11).

Optical spectra display considerable differences, depending on whether they relate to single atoms, or molecules, or larger continuous systems (e.g. solids). The difference is so large that in the spectrum of a molecule, for instance, the spectra of the indi-

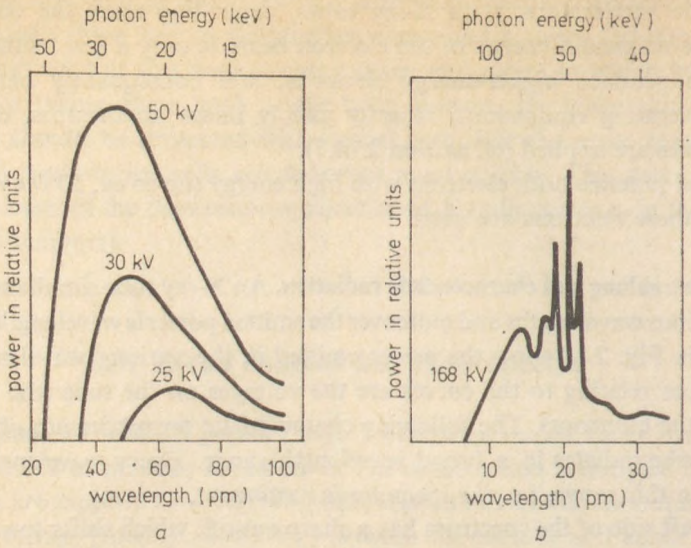

Fig. 2.18. Wavelength distribution of the emitted power for a tungsten anode
a: at lower; *b:* at higher voltages. The ordinates of the figures are not permitted to compare, namely *b* is contracted related to *a*; cf. [2.19]

vidual atoms comprising the molecule are no longer discernible. However, in X-ray spectra there is no fundamental difference between the spectra of atoms and of continuous systems (in this respect we are thinking primarily of the characteristic X-ray spectrum). The characteristic properties of the atomic spectra are conserved, and the characteristic spectra of more complex systems may be thought of to a first approximation as the summation of the spectra of the atoms. The line structure of the atomic spectra is maintained when several atoms form a more complex system, though the lines may become broadened and their positions shifted somewhat.

Obviously the structure of the X-ray spectrum is more simple and clearer than that of the optical spectrum. The main characteristic of X-ray spectra is the presence of well-separated groups of lines forming several series. From shorter towards longer wavelengths, the groups are denoted by the capital letters *K*, *L*, *M*, *N*, etc. These letters refer to the electron shells producing the spectral lines (cf. section 2.11).

The positions of some characteristic X-ray emission spectrum series on the wavelength scale are presented in Fig. 2.19. For elements of lower atomic number, only the *K* series is emitted. On increase in the atomic number of the target material, the series is shifted towards shorter wavelengths, and the *L* series, and later the *M*, and *N* series too appear.

The measurement of X-ray spectra is carried out with various devices, which are operated almost as easily as optical spectroscopes. However, whereas optical spectra are produced either by light refraction (more exactly dispersion) or by diffraction, X-ray spectra are obtained only by diffraction. The phenomenon of refraction

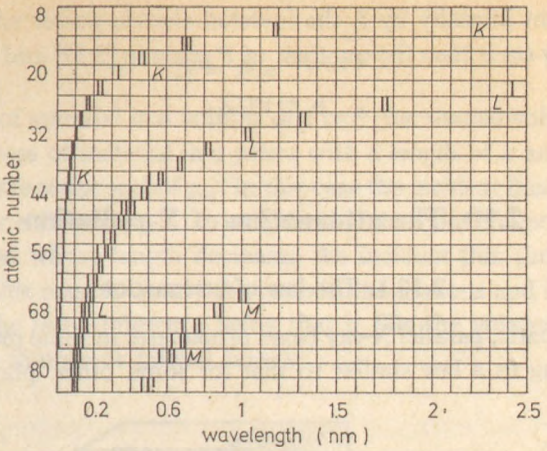

Fig. 2.19. K, L and *M* spectral series of the elements

is rather inextensive for X-rays, since at a wavelength less than 10 nm the refractive index of every material is close to 1.

4. Emitted power. The greater part of the energy of the emitted radiation, and consequently its power is due to the Bremsstrahlung. In practice, therefore, it is sufficient to discuss only this type of radiation. The following relation holds to a good approximation: the power P of the radiation is proportional to the square of the voltage U on the tube, the intensity I of electron current, and the atomic number Z of the target (anode) material:

$$P = cU^2IZ \qquad [2.19]$$

If the voltage is given in V, the current in A, and the power in W units, the value of the proportionality factor c is approximately 10^{-9}.

From the results obtained in the previous section, the following practical consequences can be drawn:

(a) An increase in the voltage results in shifts in the short wavelength cut-off and the maximum energy so that the emitted radiation becomes richer in short wavelength components, which allows regulation of its hardness.

(b) The power of the radiation increases in proportion to the intensity of the electron current, and to the square of the voltage, and consequently the intensity of the radiation too increases. If it is desired to change the intensity without varying the hardness of the radiation, only the electron current should be changed. This is attained by variation of the cathode filament heating.

With these factors in mind, the quantitative relation expressing the *efficiency* of the X-ray tube as a radiation source is found. If the voltage is denoted by U, and

the electron-current intensity by I, the invested electric power will be $P'=IU$. The degree of efficiency (η) is then the quotient of P given in [2.19] and P':

$$\eta = cUZ \qquad [2.20]$$

2.10. The attenuation of X-radiation

2.10.1. The law of attenuation

If a monochromatic, parallel X-ray beam propagates in some medium, its *intensity* decreases according to a law similar to that for some other photon radiation (cf. section 2.3.1), i.e.

$$I = I_0 e^{-\mu x}, \text{ or } I = I_0 e^{-\frac{0.693}{D} x} \qquad [2.21]$$

where μ is the attenuation coefficient, and D is the half-value thickness.

The beam may also be characterized by the *photon flux,* instead of intensity. The photon flux is defined as the number of photons flowing per unit time through unit cross-section. Let N_0 denote the photon flux penetrating into a layer of thickness x, and let N denote the photon flux passing through the layer. The following equation then holds:

$$N = N_0 e^{-\mu x}, \text{ or } N = N_0 e^{-\frac{0.693}{D} x} \qquad [2.22]$$

The value of μ depends upon the energy of radiation and the material of the medium. This latter dependence does not refer only to the fact that μ is different, for instance, for water and air; it also depends upon the density of a given substance. Accordingly, if the density of some substance changes, μ will also change. As an example, the value of μ is higher in the solid phase than in the gaseous state of the same material. With X-rays μ changes in proportion to the density (ϱ), from which it follows that the quantity

$$\mu_m = \frac{\mu}{\varrho} \qquad [2.23a]$$

called the *mass-attenuation coefficient,* is independent of the density and depends for a given substance only on the energy of the radiation. If for instance μ is measured in cm^{-1} and ϱ in $g\,cm^{-3}$, μ_m is obtained in $cm^2\,g^{-1}$. In order to distinguish μ and μ_m, the former is frequently called the *linear attenuation coefficient.*

For a clear interpretation, let us transform the exponent in [2.22]. It is obvious that $\mu x=\mu_m x_m$, where

$$x_m = \varrho x \qquad [2.23b]$$

Consequently

$$N = N_0 e^{-\mu_m x_m} \qquad [2.23c]$$

Since ϱ is the mass of material in a volume of 1 cm³ (the shaded volume in Fig. 2.20) $x_m = \varrho x$ gives the mass of material in a prism with a length of x and a cross-section of 1 cm². Let x_m be the reciprocal of μ_m. In this case the incident flux (or the intensity) decreases by a factor e. Hence, μ_m is the reciprocal of the mass of a prism, whose cross-section is 1 cm², and whose length decreases the incident flux (intensity) to $1/e$ of its original value. This mass is clearly the same in the gaseous and in the solid states of the substance, the only difference being that it fills the volume of a shorter or longer prism (i.e. a thinner or thicker layer).

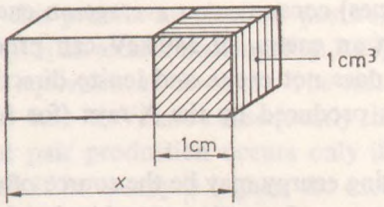

Fig. 2.20. Diagram relating to the interpretation of μ_m

Analogously to the half-value thickness one may define the half-value mass D_m. This is the mass (for a cross-section of 1 cm²) which decreases the incident flux (intensity) by half

$$D_m = \varrho D \qquad [2.23d]$$

The usual units for the half-value mass are g cm^{-2}.

2.10.2. Processes leading to attenuation

(a) **The photoelectric effect** (photoeffect, photoelectric absorption) consists of an interaction between a photon of energy $h\nu$ with one of the electrons (usually an inner shell electron) of an atom; by transferring *all of its energy* the photon is annihilated (Fig. 2.21). The energy received causes the electron (photoelectron) to rise to the surface of the atom, and with the remaining energy as kinetic energy escapes from the atom. The following energy balance applies:

$$h\nu = A + \frac{1}{2} m v^2 \qquad [2.24]$$

where $mv^2/2$ denotes the kinetic energy of the moving electron and A is the work of release necessary to raise the electron from some inner level to the atomic surface (its value for the K shell is 5–100 keV). After leaving the atom, the electron induces

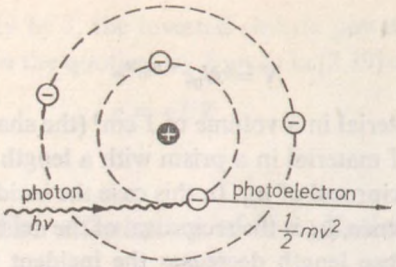

Fig. 2.21. The photoelectric effect

excitation and ionization until its excess energy is lost. The production of an ion pair in the air (or in tissues) consumes on average an energy of 34 eV, and thus a single photoelectron with an energy of 340 keV can produce 10,000 ion pairs. It follows that X-radiation does not excite and ionize directly; this is done rather by the high-energy electrons produced by the X-rays (for further details cf. sections 2.14.3 and 2.15).

The exciting and ionizing energy may be the source of numerous processes, such as heat formation, luminescence, formation of active chemical radicals, etc. However, in many cases the atoms return to their ground state by the *emission of characteristic X-radiation.* It should be noted in this connection that in some rare cases one photoelectron (every hundredth or thousandth) may suddenly be stopped in the field of an atom and, similarly to the processes in the X-ray tube, produces *Bremsstrahlung.* The series of processes continues, since the resulting characteristic radiation and Bremsstrahlung may be the source of further processes.

(b) In the **Compton effect** a photon of energy $h\nu$ again interacts with an electron, but here it transfers only *part of its energy* to the electron, and continues moving with a smaller energy $h\nu'$ in a changed direction (Fig. 2.22). In contrast with the photo-

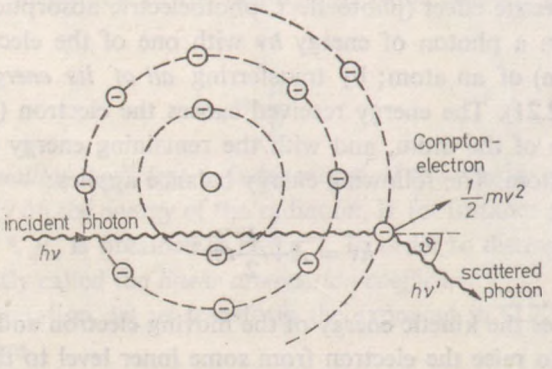

Fig. 2.22. The Compton effect

effect, the Compton effect occurs with great probability with loosely bound (or free) electrons. The process may be described by the following energy balance

$$h\nu = A + \frac{1}{2}mv^2 + h\nu' \qquad [2.25]$$

Since $h\nu'$ is smaller than $h\nu$, it follows that λ' is larger than λ, which means that this process leads to the softening of the radiation; this is the Compton scattering, the scattered electrons are Compton electrons. The change in the wavelength is independent of the wavelength of the incident photon and depends only on the direction (ϑ) of the scattering.

The fate of the Compton electrons is subsequently similar to that of photoelectrons, and the scattered photons behave in the same way as other photons.

(c) **Pair production.** In this process an electron–positron pair is produced by an X-ray photon in the vicinity of an atomic nucleus (Fig. 2.23). The process is governed by Einstein's energy-mass equivalence: $E=mc^2$. The rest mass of an electron (or a positron) is equivalent to 0.51 MeV, and consequently that of one pair to roughly 1.0 MeV. This means that pair production occurs only if the energy of an X-ray photon is at least 1.0 MeV. If its energy is higher, the excess appears in the kinetic

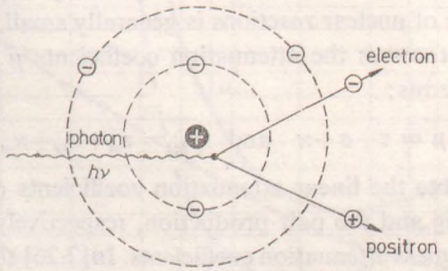

Fig. 2.23. Pair production

energies of the electron and the positron. The components of the pair subsequently produce ionization and excitation in the same way as photoelectrons or Compton electrons with the corresponding energy. On slowing down, the positron component unites with an electron, their encounter leading to their annihilation, generally with the emission of two γ-photons. The γ-photons induce the same effects as X-ray photons of equivalent frequency.

(d) **Coherent or classical scattering.** The above three processes result in the total or partial transformation of the photon energy. However, the photon sometimes changes only its direction, without energy loss. This type of coherent (classical) scattering occurs mainly on electrons; the scattering on atomic nuclei is negligible compared with that on electrons.

(e) **Nuclear reactions.** High-energy photons may interact with atomic nuclei. The total energy is transferred to the nucleus, more exactly to a nucleon in it. This

nucleon may then have sufficient energy to escape from the nucleus. In most cases a neutron is released; proton release is rare. The binding energy per nucleon in the different nuclei is about 7–8 MeV. Nuclear reactions can be produced only with X-rays having a higher photon energy than this. With two of the lightest elements in the periodic system (heavy hydrogen and beryllium), however, a photon energy of even a few MeV is sufficient to induce nuclear transformations: the nucleus absorbs a photon and releases neutron. In this way a new nucleus is produced, and the released neutrons readily give rise to further nuclear reactions.

2.10.3. Attenuation (absorption) spectra

The processes discussed above do not occur with equal probability. At low photon energies (especially with elements of high atomic numbers) the photoelectric effect prevails; with increasing energy the probability of the photoeffect decreases, and (especially with elements of low atomic numbers) the Compton effect comes into prominence. The coherent scattering is appreciable only in the case of low photon energies (below 50 keV), but even here it is merely 6–10% of the Compton scattering. Above 1 MeV, pair production too assumes increasing importance. Besides these effects, the probability of nuclear reactions is generally small.

For the different processes the attenuation coefficients μ and μ_m are considered to consist of several terms:

$$\mu = \tau + \sigma + \varkappa \quad \text{and} \quad \mu_m = \tau_m + \sigma_m + \varkappa_m \qquad [2.26]$$

where τ, σ and $\varkappa$ denote the linear attenuation coefficients due to the photoeffect, the Compton scattering and the pair production, respectively, while τ_m, σ_m and $\varkappa_m$ refer to the respective mass attenuation coefficients. In [2.26] the nuclear reactions are usually neglected, and the classical scattering is considered together with the Compton effect. However, if the radiation is sufficiently hard, compared with the Compton scattering the classical scattering is disregarded.

The values of the attenuation coefficients depend on the material of the medium and the energy of the photons. As an example, the values for lead are presented. Figure 2.24 refers to lower, and Fig. 2.25 to higher photon energy. The former presents the absorption spectrum produced only by the predominant photoelectric effect (absorption spectrum in the narrower sense), while the latter depicts not only the resulting attenuation spectrum, but also the component spectra. Figure 2.25 illustrates the above observations relating to the courses of the component spectra too.

From Figs 2.24, 2.26 and 2.27, further conclusions may be drawn with regard to the attenuation (absorption) of X-radiation.

(a) Not only the emission, but also the absorption spectra are more simple in the X-ray than in the optical range. Thus, for instance, it is striking in Fig. 2.24 that at

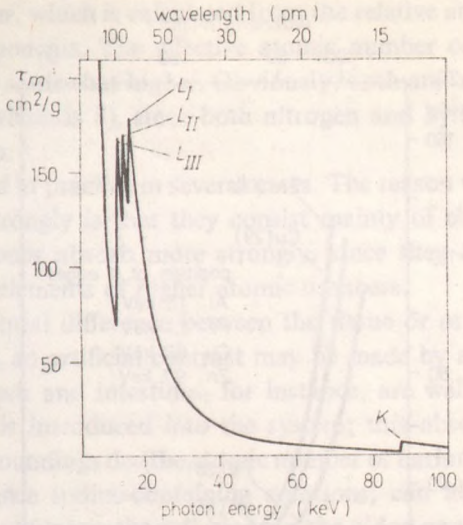

Fig. 2.24. Absorption spectrum of lead

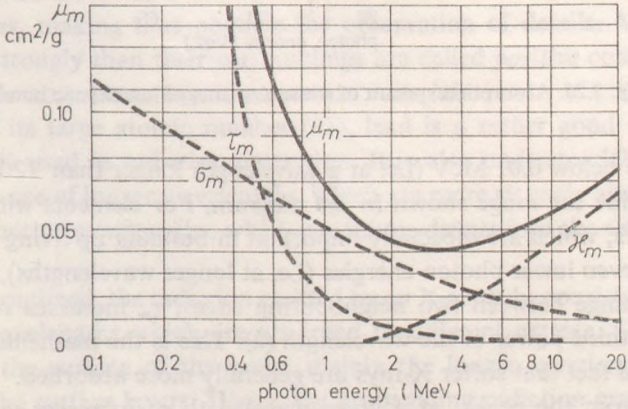

Fig. 2.25. Total and partial attenuation coefficients of X-rays as functions of photon energy, for lead
Logarithmic scale on the abscissa

certain wavelengths the curve displays peaks, called *absorption edges*. Similarly as for the emission lines, these edges form groups, and are appearing in about the same energy range as the emission lines. The edges are also denoted by capital letters *K, L, M*, etc. on proceeding from higher to lower energy values (i.e. from shorter to longer wavelengths). The spectrum shows the single *K* edge and the *L* triplet of lead (lower-energy groups, including the *M* edges, are not depicted).

(b) Figure 2.26 shows the absorption spectra of some elements with low atomic numbers. Neither edges nor peaks are to be seen in the curves; *K edge with highest*

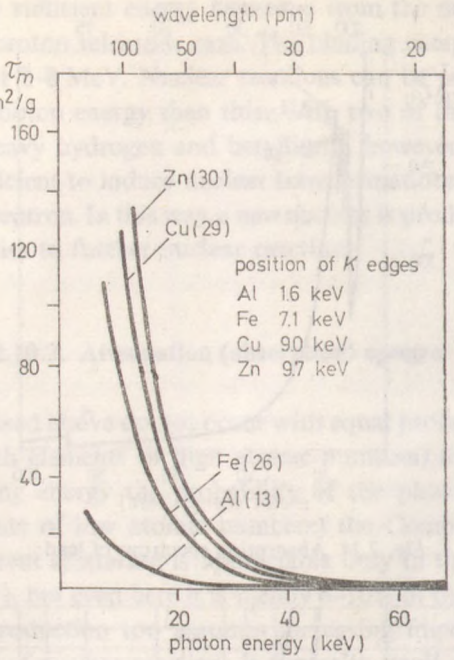

Fig. 2.26. Absorption spectra of some elements of low atomic number

energy appears below 0.01 MeV (i.e. at a wavelength longer than 120 pm), and consequently outside the range shown in the diagram. For elements with even smaller atomic numbers, which are especially important in building up living organisms, the K edges lie at even lower photon energies (i.e. at longer wavelengths).

(c) In the range between two neighbouring edges, τ_m increases roughly in proportion to the third power of the wavelength (λ). This is the mathematical reflection of the empirical fact that softer X-rays are generally more absorbed.

(d) Apart from the peaks, at a given wavelength τ_m increases with the atomic number Z of the element; the increase is roughly proportional to the third power of the atomic number. This expresses the important empirical fact that a given radiation is absorbed more strongly by elements of higher than of lower atomic number. This may be generalized: absorbents which are richer in elements of higher atomic number absorb the radiation more strongly than absorbents composed of substances of lower atomic numbers.

The results of points (c) and (d) can be given in a single relation:

$$\tau_m = C\lambda^3 Z^3 \qquad [2.27]$$

The value of the proportionality factor C on the short-wave side of the K edge is 5.5–6.5 (if λ is measured in nm and τ_m in $cm^2\,g^{-1}$). If not elements but complex substances are investigated, Z is replaced by the mean atomic number, also called the

effective atomic number, which is calculated from the relative amounts and the atomic numbers of the components. The effective atomic number of air is approximately 7.3, and that of water somewhat higher. Obviously, both are smaller than the atomic number of oxygen (which is 8), since both nitrogen and hydrogen precede oxygen in the periodic system.

(e) [2.27] is applied in practice in several cases. The reason why soft tissues do not absorb X-ray very strongly is that they consist mainly of elements of low atomic numbers, whereas bones absorb more strongly, since they also contain relatively large proportions of elements of higher atomic numbers.

If there is no essential difference between the tissue or organ to be investigated and its surroundings, an artificial contrast may be made by applying some *contrast substance*. The stomach and intestines, for instance, are well outlined if a barium sulphate suspension is introduced into the system; this absorbs X-radiation more strongly than the surroundings do (the atomic number of barium is 56). *Liquid* contrast substances, for instance iodine-containing solutions, can also be used to obtain contrast images of the kidneys, the gall-bladder, the blood vessels, etc., which thereby become well outlined (the atomic number of iodine is 53). In some cases *gaseous* contrast materials are used. Their low density means that they absorb less strongly than the tissues, making thus possible the observation of details. Materials which absorb more strongly than their surroundings are called *positive* contrast materials, and the weaker absorbents *negative contrast materials*.

Because of its large atomic number (83), lead is a rather good absorbent. For this reason it is used in radiation protection. It is also understandable why surface therapy makes use of longer wavelengths, which are more strongly absorbed, whereas radiation of shorter wavelengths, which penetrates deeper into the tissues, is used in deep therapy.

As already outlined, the radiation emitted by an X-ray tube consists of components of different wavelengths which are absorbed to different extents. Of the radiation encountering the surface of the body, mainly the longer wavelength radiation is absorbed by the surface layers. Hence, the propagating radiation gradually becomes poorer in softer components and richer in harder components. In therapeutic treatment the softer radiation absorbed in large quantities by the surface layers (e.g. the skin) may cause undesired damage; in order to avoid this, the softer radiation is filtered out with intermediate metal plates (copper, aluminium, etc.; Table 2.2). *The filters* also attenuate the stronger components, but to a lesser extent than the softer ones. As a consequence, the radiation falling on the body will be more homogeneous, and at the same time the damage to the surface layer will be decreased.

(f) Figure 2.27 depicts the absorption curves of water and air. The soft tissues of the body absorb in practically the same way as water, and consequently the data relating to the water curve are also valid for the tissues.

It should be noted that the curves for air and water are nearly parallel and the ratio of their absorption constants is practically the same throughout the whole range.

Table 2.2

Half-value thicknesses of some substances

Wave-length (pm)	Photon energy (keV)	Half-value thickness (cm)				
		Air (standard) state)	Water	Al	Cu	Pb
10	124	3800	4.55	1.72	0.25	0.017
30	41	2150	2.9	0.55	0.017	0.0044
50	25	1120	1.45	0.122	0.0042	0.0010
100	12	205	0.25	0.017	0.00061	0.000077
200	6	25.5	0.033	0.0024	0.000071	0.000012

This fact plays an important role in *X-ray dosimetry*. The X-radiation absorbed in the air is relatively easily measured, in contrast with the radiation absorbed by water or by tissues (cf. section 2.16.1). From the similarity of the curves it follows that the energy absorbed by the tissues at some site may be considered proportional to the energy absorbed by the air at the same place, and what is even more essential, the proportionality factor is the same in the wavelength range of practical importance. Consequently, it is sufficient to measure the energy absorbed by the air, for if this is found to be higher by a factor of k times, then the tissues at the same site will

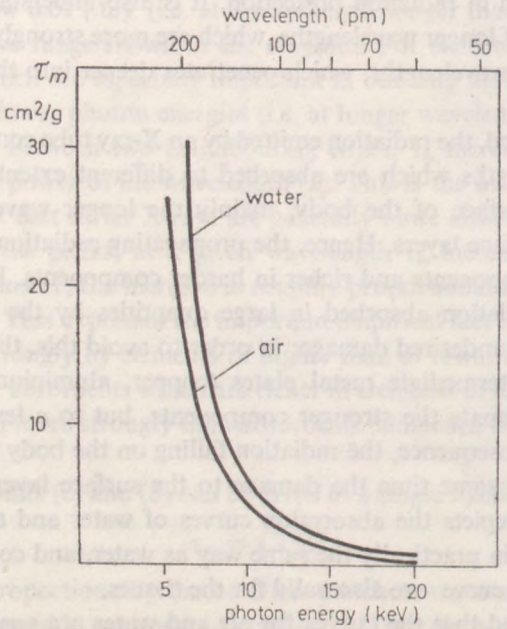

Fig. 2.27. Absorption spectra of air and water

also absorb k times more energy, regardless of whether the X-ray spectrum is richer in harder or in softer components.

The attenuation of X-radiation has been seen to result from *scattering* too (classical or Compton scattering). The mass scattering coefficient for light elements in the range generally used in medical practice is nearly independent of the wavelength; its value is ca. 0.2 $cm^2\ g^{-1}$, except for hydrogen, which has a value of ca. 0.4 $cm^2\ g^{-1}$. The mass scattering coefficient of the body tissues is close to 0.3 $cm^2\ g^{-1}$. In practice the scattering is never negligible. It must be taken into account in the determination of the dose when radiation is used for therapeutic purposes, and it must never be neglected when the protection of the health of the medical staff is concerned.

2.11. Interpretation of X-ray spectra

1. Origin of characteristic radiation. Whereas the optical spectrum provides information about the changes in the state of the outer electrons, also called optical electrons, and thus about processes occurring in the outer energy levels, the characteristic X-ray spectra shed light on the processes within the inner electron shells. This situation is presented in Fig. 2.28, which shows as an example the energy levels of the copper atom. The diagram is only an outline, since every principal quantum number, $n=1, 2, 3, \ldots$ is represented by only one level, though they actually consist of several levels close to one another (see section 1.2.2). Consequently, every line represents a group of energy levels. Optical spectra are generated by exciting the *outer* electrons from the state with principal quantum number $n=4$ into some higher unoccupied state (possibly in the continuum), after which the electron returns in one or more steps to the level of the principal quantum number $n=4$. X-radiation,

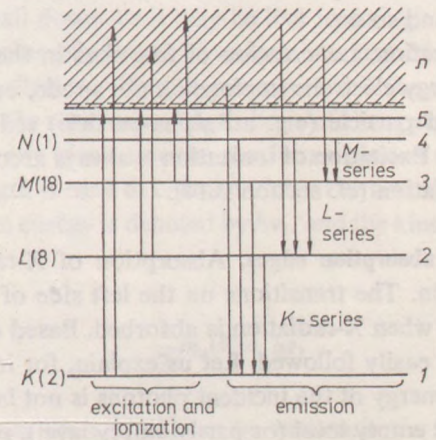

Fig. 2.28. Simplified energy level system of the copper atom
The numbers in brackets are the numbers of electrons in the individual shells

however, is generated if some of the electrons of the K, L or M shells are excited to higher levels or into the continuum (this process is represented in the diagram by upward-pointing arrows), and electrons in higher energy state return to the holes generated by the excitation in these shells (downward-pointing arrows). The K series is produced by the transitions whose final state is the K state, while the L series and the M series are the transitions to the L and M states, respectively. The whole picture is reminiscent of the generation of the hydrogen atom spectrum and the various series can be described with relations similar to the Lyman, Balmer, etc. series. The similarity also exists insofar as every series is joined by a continuum.

In order for X-radiation to be produced, the creation of free sites or holes in the inner shells is necessary. Until this occurs, the inner shells cannot accommodate electrons, since according to Pauli's principle they are completely filled. Consequently, it is impossible to excite only optical electrons so that they are moved, say, into the K shell instead of some outer (partly or fully) empty level. In the same way it follows that an excited K shell electron cannot move directly into the filled L shell above the K level. This transition can occur only if there is an empty site (hole) in the L shell, but this is highly improbable. This explains why the arrows indicative of excitation without exception point upwards to the upper empty levels, i.e. to the continuum in the diagram.

The differences between the inner energy levels are much larger than those in the optical range, and hence the energy of X-ray photons is much larger than the energy of optical photons. This train of thought makes it obvious that elements whose electrons occupy more and more levels with increasing atomic number yield an increasing number of series, which in turn are increasingly richer in lines, and the spectra become shifted towards higher energy values. In the case of hydrogen and helium, which have no inner shells, there is no X-ray spectrum, but only an optical one. From lithium to neon, only the K series is present; from sodium to argon, the L series also appears; and so on.

Excitation and ionization, i.e. creation of free sites in the inner shells, occur in X-ray tubes if high-energy electrons impinge on the anode, but excited states can be created by any charged particle (e.g. an alpha-particle) striking some target with sufficiently high energy. Excitation of ionization is always accompanied by the release of characteristic X-radiation (cf. section 2.14).

2. Interpretation of absorption edges. Absorption of X-radiation also produces excitation and ionization. The transitions on the left side of Fig. 2.28 demonstrate the processes occurring when X-radiation is absorbed. Based on it the appearance of the absorption edges is easily followed. Let us explain, for instance, the L edge (or edges). As long as the energy of the incident photons is not large enough to raise an L electron to the lowest empty level (or partly empty level), no absorption occurs in the L shell. If the photon energy is large enough to produce this transition however, there is some probability that the atom will absorb the photon, and the absorption

suddenly increases. This increase in absorption produces the L absorption edges. Photons whose energy is larger than the limiting energy may be absorbed too, though the probability of absorption decreases with increasing energy. For this reason, on passing from the L edges to the K edge the absorption constant decreases; however, it increases again if the photon energy is sufficiently high to raise the electron from the K shell. The occurrence of multiple edges is connected with the fact that several energy levels belong to the same shell, and excitations may occur in any of them. Each of them gives rise to individual absorption edges, but these are situated close to one another (cf. section 2.10.3).

3. Interpretation of Bremsstrahlung. So far, the only electron transitions considered have been those in which the initial or final state (possibly both) belong to some *discrete* energy level. However, transitions also exist in which both the initial and final states belong to the *continuous* energy range (the continuum). In this case free electrons pass over from one state into another. Two possibilities exist: the free electron returns from a higher to a lower state, or conversely the transition is from a lower to a higher energy state. In the first case the electron becomes decelerated in the atomic or ionic force field, while in the second it is accelerated. The first case may be accompanied by the emission of radiation, whereas in the second case radiation may be absorbed. Since both the initial and the final states now lie in the continuous energy range, the spectrum will be continuous.

From the practical viewpoint, especially emission spectra induced by the deceleration of high-velocity electrons in the force field of an atom or ion are important. The radiation produced is the Bremsstrahlung and its spectrum is continuous (cf. Fig. 2.18). This process occurs, for instance, in X-ray tubes when accelerated electrons are decelerated in the force field of the atoms of the anticathode.

The decelerated electrons lose various amounts of energy. The energy may be lost step by step in small doses, or it may be lost in a single act. The various energy losses result in the emission of photons of various energies. Maximum-energy photons, i.e. photons of the smallest wavelength, are emitted if the electrons lose their total energy in a single act; the short-wavelength limit of the spectrum in Fig. 2.18 can be interpreted by this. In any given case the maximum photon energy corresponding to the minimum wavelength is easy to calculate from the law of conservation of energy. If the maximum photon energy is denoted by $h\nu_l$, and the kinetic energy of the impinging electron is $m_e v^2/2$:

$$\frac{1}{2} m_e v^2 = h\nu_l \qquad [2.28]$$

where ν_l is the frequency limit. [2.28] can be written in the form used in practice by expressing the kinetic energy in terms of the accelerating voltage U ($m_e v^2/2 = eU$), and introducing the limiting λ_l wavelength ($\nu_l = c/\lambda_l$) instead of the limiting frequency.

Substituting into [2.28] leads to

$$\lambda_l = \frac{hc}{eU} \qquad [2.29]$$

[2.29] is known as the *Duane–Hunt law* in radiology, for the relation between U and λ_l was carefully measured by these authors.

2.12. Some problems of X-ray diagnostic image formation

The production of X-ray images presents some theoretical and numerous practical problems. In the following section the details will not be considered, and only a few of the essential questions will be dealt with.

1. Summation images and tomograms. The traditional X-ray image is a simple shadow image. Darker and brighter image details are produced on the screen, the contrast depending upon the extents of absorption of the tissues in the path of the radiation beam. The simple shadow image does not yield information separately about the details of the spots at different depths and absorbing to different degrees. The shadows of the successive layers appear together, producing the *summation image*.

The separate investigation of the various layers at different depths requires special methods. The essence of the production of traditional layer images (tomography, planigraphy, stratigraphy) is depicted in Fig. 2.29. The enclosed area represents the cross-section of the body (for instance, a thoracic section perpendicular to the axis of the body). The examined layer perpendicular to the plane of the diagram is represented by the line MN. The radiation source and the detector (X-ray film) move synchronously in opposite directions during the exposure. The position of the rotation axis (O) of the displacement is selected so that its extension is situated on the examined layer. It may be observed that, while the radiation source moves from S_1 to S_2, the shadow of the point O moves from O_1 to O_2, and the shadows of the points A and B move from A_1 to A_2, and from B_1 to B_2 respectively. Simple geometrical considerations show that the shadow of every point on the examined layer is displaced to the same extent, i.e. for instance, $\overline{O_1O_2}=\overline{A_1A_2}$. However, in comparison with these points the points above the layer are displaced to a larger extent, whereas the points below the layer are moved to a smaller extent. It follows that the shadow of the points of the examined layer on the film displaced by $\overline{O_1O_2}=\overline{A_1A_2}$ remain in their position, whereas the shadow of every other point becomes blurred. In principle sharp images are obtained only from an infinitely thin layer, but in practice even 1–2 mm thick layers yield satisfactory results. The information acquired is made more complete, if X-ray pictures are taken of more layers above and below each other, simply by changing the position of the axis of rotation with respect to the body.

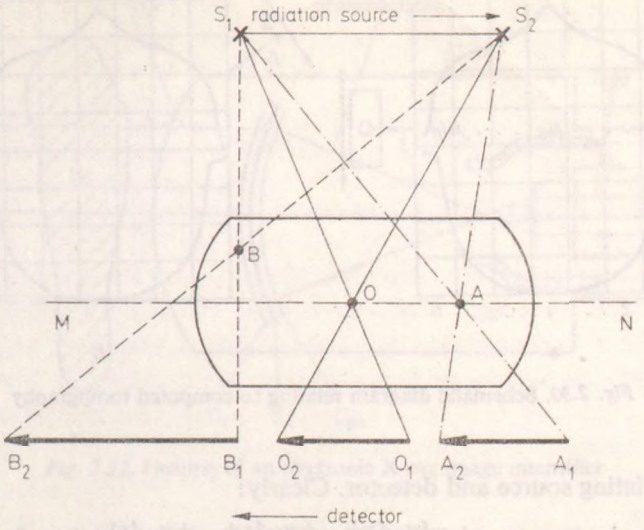

Fig. 2.29. Schematic diagram relating to traditional tomography

2. Computed tomography. The appearance and application of computers led to qualitatively new solutions via the development of *X-ray densitography*. This method is also called *computed tomography* (abbreviated as CT). In practice increasingly more sophisticated equipment types are being marketed, but since the principles of their operation are the same, only these principles are discussed below.

Let us consider a cross-sectional layer, for instance a 1.0 mm thick skull section, perpendicular to the axis of the body. Let the layer be covered by a network of 1.0 mm^2 squares. If a 20×30 cm^2 surface is assumed, the network contains 60,000 elements. This situation is outlined in Fig. 2.30. Valuable information on the layer is obtained if the attenuation coefficient of every element of the net, the *density matrix*, is known. Since the attenuation coefficients of the various tissues are different, the differences in attenuation of the healthy and pathological tissues may be used for diagnostic purposes. CT is based on these quantities, and the layer image displayed on the screen of a cathode-ray tube consists of elements of the *image matrix* differing from one another in colour or in the degree of grey tone, according to the different density values.

Figure 2.30 also presents the more important phases of the image production. The layer positioned for image formation is transirradiated by a narrow X-ray beam approximately 1.0 mm in cross-section from the source (S). The source moves past the layer. On the opposite side the detector (D, e.g. a scintillator crystal), which measures the intensity of the emerging radiation, moves together with the source in approximately 1 mm steps (or continuously). It can be seen in Fig. 2.30*a* that a row of the density matrix participates in the intensity decrease relating to the momentary posi-

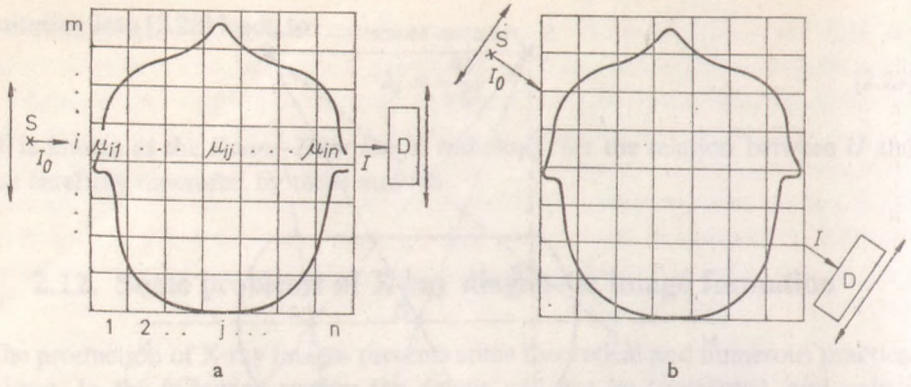

Fig. 2.30. Schematic diagram relating to computed tomography

tion of the radiating source and detector. Clearly:

$$I = I_0 e^{-(\mu_{i1}\Delta x + \ldots + \mu_{ij}\Delta x + \ldots + \mu_{in}\Delta x)} \qquad [2.30]$$

where I_0 is the incident, and I the emerging intensity, Δx represents the thickness of the matrix element, and $\mu_{i1}, \ldots \mu_{ij}, \ldots \mu_{in}$ are attenuation coefficients characteristic of the individual elements (in our example the number of elements in a row of the matrix $n=200$). Similar relations apply to every matrix row (the number of rows $m=300$). Overall, a knowledge of mn, i.e. 60,000 elements, is required for the example discussed. A one-directional displacement of the radiation source and the detector along the layer is clearly insufficient, since this would furnish only 300 equations in our example. However, the problem is solved by the equipment scanning the same sectional layer in several directions. Fig. 2.30*b* demonstrates the case of scanning in a position rotated by 60 degrees with respect to the first one. After scanning in a sufficiently large number of directions (e.g. one-degree rotations carried out automatically by the equipment), the computer calculates from the stored data the density of the matrix elements, and simultaneously codes the results according to colour or to grey tone, and displays the image of the section, i.e. the densitogram (Fig. 2.31 in the Supplement).

3. Electronic image intensifier. It is important to decrease the radiation hazard to which the patient and the medical personnel may be exposed. This protection is quite effectively achieved with an electronic image intensifier, whose operating principle is demonstrated in Fig. 2.32. The X-radiation propagating through the transilluminated part of the body (*TB*) falls onto a luminescent screen (E_1) connected with a photocathode (C_{ph}), both placed in a vacuum tube. The screen and the photocathode are supported by a thin aluminium plate (C) through which the radiation strikes the screen with practically no attenuation. The luminescent light removes electrons from the photocathode; these are accelerated in an electric field. The accelerating voltage

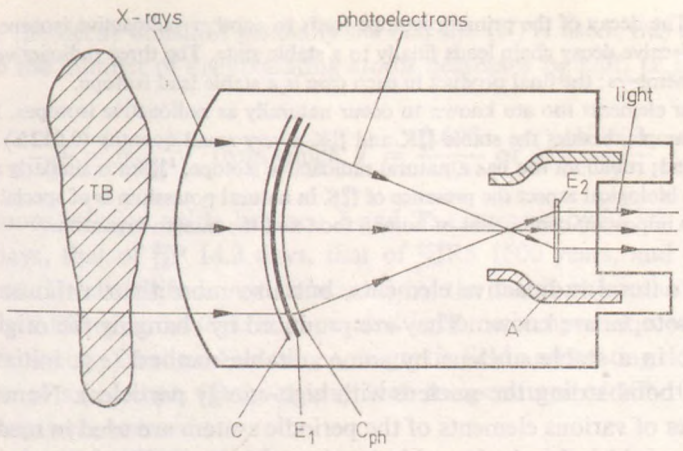

Fig. 2.32. Outline of an electronic X-ray image intensifier

(10–50 kV) is placed across the aluminium plate serving as cathode and the anode (A). The different domains of the photocathode emit different electron quantities. The number of these electrons depends from point to point on the intensity of the generated light on the luminescent screen. The accelerated electrons emerging from the photocathode and passing through an appropriate electric field (electric lens) produce a reduced, real image on the luminescent screen E_2. The method is similar to that in the electron microscope. With this set-up the same image appears on the screen E_2 as on E_1, but much more brightly. The brightness increase, i.e. the image intensification is caused by the acceleration of photoelectrons. The image produced on screen E_2 is observed through an optical magnifying glass. It is so bright that certain manipulations (e.g. the repositioning of fractured bones or sprains) can be controlled in the course of operation even in illuminated rooms. The great brightness allows photographic series or even motion pictures of the image produced. Moreover, the optical magnifying system may be replaced by a television camera to transmit the X-ray image to any distance.

2.13. Radioactive isotopes. The decay law. Biological half-life

1. Radioactive isotopes. Certain atoms (isotopes) are unstable and their nucleus disintegrates with the emission of some particle. Such atoms are *radioactive atoms (radioactive isotopes)*. Numerous radioactive isotopes occur naturally, mainly among the heavy atoms at the end of the periodic table (e.g. uranium, thorium, actinium).

The *natural* radioactive isotopes of the heavy elements belong to the uranium, thorium and actinium families. Each family is headed by a long-living primary isotope: at the head of the thorium series is $^{232}_{90}$Th, at that of the actinium series is $^{235}_{92}$U, and at that of the uranium–radium

series is $^{238}_{92}U$. The decay of the primary isotope leads to another radioactive isotope which decays further; the extensive decay chain leads finally to a stable state. The three radioactive families contain about 44 members; the final product in each case is a stable lead isotope.

Some lighter elements too are known to occur naturally as radioactive isotopes. In natural potassium, for example, besides the stable $^{39}_{19}K$ and $^{41}_{19}K$ a very small quantity (0.012%) of radioactive $^{40}_{19}K$ can be found; rubidium too has a natural radioactive isotope. $^{147}_{62}Sm$ is similarly radioactive by nature. From a biological aspect the presence of $^{40}_{19}K$ in natural potassium is of special interest, since potassium is an important component of human food and the human organism.

Not only natural radioactive elements, but also more than a thousand *artificial* radioactive isotopes are known. They are produced by changing the original proton–neutron ratio in a stable nucleus by some suitable method (e.g. initiating nuclear processes by bombarding the nucleus with high-energy particles). Numerous radioactive isotopes of various elements of the periodic system are used in medical science. For instance, besides the single stable isotope of iodine ($^{127}_{53}I$) more than ten radioactive iodine isotopes exist; the radioactive variants of sodium ($^{22}_{11}Na$, $^{24}_{11}Na$) are also frequently used.

Since the radioactive decay and connected processes of natural and artificial radioactive elements are governed by the same laws, no differences will be made between them in the following sections.

2. The decay law. Consider a radioactive preparation containing N radioactive atoms of the same kind. Each of them possesses an identical excess energy, but nevertheless their decay does not occur simultaneously. It cannot be stated with certainty at what time a given atom will decay. However, if sufficient atoms are involved, the question of how many of them will decay in a given time can be answered. The *decay number per unit time,* or more exactly the decay rate dN/dt, *is proportional to the number of undecayed atoms at a given time:*

$$\frac{dN}{dt} = -\lambda N \qquad [2.31]$$

The proportionality factor λ is different for the different atoms, but it is constant (the *decay constant*) for the same kind of atoms. According to [2.31] λ defines the fraction of the total number of atoms which decay in unit time. If, for instance, $\lambda = 1\ h^{-1}$, 1/3600 of the total number of atoms disintegrate per second. (The negative sign indicates that the number of radioactive atoms decreases in time.) Integration of [2.31] gives

$$N = N_0 e^{-\lambda t} \qquad [2.32]$$

where N_0 denotes the number of undecayed atoms at $t=0$ (at the beginning of the observation), and N is the number of undecayed atoms at time t. [2.32] indicates that the decay of radioactive substances is governed by an exponential law; $N=0$ at $t=\infty$, which means that in principle a given radioactive substance disintegrates totally only in an infinite time.

Instead of the decay constant generally *the half-life* (T) is used; this is the period during which the number of disintegrating atoms decreases by half. [2.32] can be rewritten:

$$\frac{N_0}{2} = N_0 e^{-\lambda T} \text{ from which } T = \frac{0.693}{\lambda} \text{ or } \lambda = \frac{0.693}{T} \qquad [2.33]$$

An unambiguous relation exists between λ and T, expressed by [2.33]. The half-life of $^{131}_{53}I$ is 8 days, that of $^{32}_{15}P$ 14.3 days, that of $^{222}_{88}Ra$ 1600 years, and that of $^{238}_{92}U$ 4.5×10^9 years. The half-life is not influenced by the usual physical and chemical effects. The half-life of an isotope incorporated in some compound is identical with that of the elementary isotope. Pressure, a magnetic field and heating do not change the decay rate of an isotope. (Only at very high temperatures of 10^4–10^5 K is some detectable deviation observed.)

With the use of the half-life, the decay law can be reformulated to give

$$N = N_0 e^{-\frac{0.693}{T}t} \qquad [2.34]$$

The mean life-time (τ) is the time in which the number of undecayed atoms decreases by a factor e. Straightforward calculation yields

$$\tau = \frac{1}{\lambda} = 1.443T \qquad [2.35]$$

3. Biological half-life. The quantity of a radioactive isotope introduced into the organism and distributed either uniformly or enriched in some organ or tissue decreases not only because of the physical disintegration, but also as a result of the *biological* elimination characteristic of the isotope as a chemical element. Consequently, if the isotope content of the organism or an organ is measured continuously, a half-life shorter than that expected from the physical decay is obtained. The resultant of the physical decay and the biological decrease is the *effective half-life*. The physical half-life (T_{phys}), the biological half-life (T_{biol}) and the effective half-life (T_{eft}) are connected by the relation

$$\frac{1}{T_{eff}} = \frac{1}{T_{phys}} + \frac{1}{T_{biol}} \qquad [2.36]$$

Since T_{phys} and T_{eff} are directly measurable, T_{biol} can be calculated from [2.36]. The biological *half-life* is defined as the time in which half the quantity of the element or compound in the organism, organ or tissue is eliminated by biological processes.

The biological half-life is independent of whether the isotope is stable or radioactive since stable and radioactive isotopes behave similarly in the life processes. However, until radioactive isotopes became available, the simple and exact measurement of the biological half-life was not possible. The radiation of an administered radioactive isotope makes this isotope distinguishable from the stable isotope already

present in the organism. As an example $^{131}_{53}$I has a physical half-life of 8 days, but when present in the thyroid gland it has an effective half-life of 7.5 days. From [2.36] the biological half-life is therefore 120 days. This is the time normally necessary to remove half the iodine present in the thyroid gland. Of course, the eliminated quantity is continuously replaced.

Once in the body, radioactive isotopes may become enriched in certain organs, the *critical organs* (e.g. the liver). Consequently, careful attention must be given to protecting these organs from hazardous radiation effects. The thyroid gland is the critical organ for iodine. The critical organ for $^{51}_{24}$Cr is the kidney. $^{24}_{11}$Na is fairly evenly distributed in the extracellular fluid, and consequently the whole organism is considered to be critical for this isotope. These or other organs (e.g. the liver) are also considered to be critical, if their functions are especially important for the normal functioning of the whole organism.

4. The activity of a radioactive substance is characterized by the decay rate, which is the decay number per second. The unit of activity is the becquerel (denoted by Bq):

$$1\ \text{Bq} = 1\ \text{decay/s}, \quad \text{or} \quad 1\ \text{Bq} = 1\ \text{s}^{-1}$$

Previously the curie (denoted by Ci) was used:

$$1\ \text{Ci} = 3.7 \times 10^{10}\ \text{Bq}$$

Since the decay rate is proportional to the number of undecayed atoms present, the decay law [2.34] may be used for the change in time of the activity:

$$A = A_0 e^{-\frac{0.693}{T} t} \qquad [2.37]$$

where A_0 is the initial activity of the preparation, and A is the activity still present after time t.

The concept of *specific activity* is frequently used. This denotes the activity as related to unit mass. Its unit is Bq/kg.

In a broader sense the notion of specific activity is associated not only with a pure radioactive substance but also with its mixture with e.g. the same inactive substance (the *carrier substance*[4]); moreover, the specific activity of liquids or tissues is sometimes also used. The activity related to unit volume is the radioactive concentration; its unit is Bq/l.

[4] In most samples to be administered the mass of the radioactive isotope is extremely small, e.g. the mass of 37 MBq (1 mCi) $^{131}_{53}$I is 8.1×10^{-9} g, and the mass of $^{24}_{11}$Na is only 0.113×10^{-9} g. For administration, these small quantities of radioactive compounds are generally mixed with some other substance (usually the same, inactive compound); this is the carrier substance.

2.14. Nuclear radiation and its applications

Radioactive nuclei may release their excess energy in various ways. In a considerable number of cases this energy or part of it is carried off by some particle. In these cases the nuclear transformation is accompanied by corpuscular radiation. However, the release of excess energy may also produce electromagnetic radiation, and it is an even more frequent occurrence that part of the excess energy is released in the form of corpuscular radiation (of course, the energy released also includes the energy proportional to the escaping mass), and the remaining excess energy is then emitted as electromagnetic radiation. Though not belonging strictly to radioactive phenomena, the processes of *nuclear fission* and *spallation* may be mentioned here. The essence of the former effect is the fission of certain (mainly heavy) nuclei into two parts of comparable mass *(fission products)*. Nuclear fission was first observed with $^{235}_{92}U$; besides being radioactive this isotope can capture a slow neutron and then undergoes fission into two medium heavy nuclei, at the same time emitting 2 or 3 neutrons. *Nuclear spallation* is induced by extremely high-energy alpha, deuteron or similar radiation. A nucleus bombarded in this way readily disintegrates into smaller nuclei, nuclear fragments, protons and neutrons.

2.14.1. Alpha-radiation (α-radiation)

1. α-decay, α-particles. In the course of α-decay an atomic nucleus releases a high-energy *helium nucleus,* the *α-particle*. As a result, the atomic number of the nucleus decreases by 2, and its mass by 4. For instance:

$$^{226}_{88}Ra \rightarrow {}^{222}_{86}Rn + {}^{4}_{2}\alpha \qquad [2.38]$$

2. The interaction of α-radiation with matter. The initial velocity of α-particles is several thousand km/s, which is equivalent to a kinetic energy of several MeV. The energy of these particles is lost through ionization or excitation of the molecules and atoms of the surrounding medium, for instance the air. The ionizing power of an α-particle is characterized by the *linear ion density (specific ionization)* produced along the particle path. If the particle produces n ion-pairs along a track of length l, the linear ion density is given by the quotient n/l; this is the number of ion-pairs produced per unit track length. The linear ion density of α-particles is 20,000–80,000 ion-pairs per cm in air at normal atmospheric pressure. The production of one ion pair in air requires an energy of 34 eV. If the velocity of an α-particle decreases to the thermal value it is transformed into a helium atom by the capture of 2 electrons.

The path of an α-particle is straight except for the rare cases when the α-particle interacts not with an electron shell, but with a nucleus, which is small even compared to the electron shell. In this case the α-particle is scattered on the nucleus. The mass of the α-particle is considerably (approximately 7000 times) larger than that of the

electron, which explains why a collision with electrons does not influence the direction of the motion of α-particles. *The effective range R* is the distance covered by an α-particle in a medium of density ϱ until its energy E decreases to its thermal value. For substances consisting of elements of low atomic numbers (e.g. air, water, the body tissues) the following relation holds to a good approximation

$$R = k\frac{E^{3/2}}{\varrho} \qquad [2.39]$$

If E is measured in MeV, ϱ in g cm^{-3} and R in cm, the numerical value of k is $4.15\times$ $\times 10^{-4}$. For instance, in atmospheric air for Ra (E=4.8 MeV) R=3.4 cm. In liquids or soft tissues the effective range of an α-particle is 10–100 μm.

The ionizing power of an α-particle is almost constant at the beginning of its path, but towards the end increases about fourfold, and attains the above value of approximately 80,000 ion-pairs/cm, from which it falls abruptly to zero as shown in Fig. 2.33. Thus, the linear ion density is smaller with high-energy particles, and increases with decreasing energy.

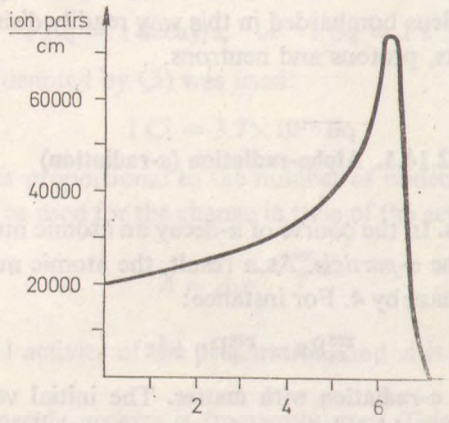

Fig. 2.33. Specific ionization of the ^{214}Po α-particle as a function of its track length (in air)

The behaviour of α-particles in different media is usually characterized by the *stopping power* of the medium, which is defined by the energy loss of the α-particle per unit track length. Instead of the stopping power, the expression *linear energy transfer* (LET) is frequently used. By definition, the stopping power is equal to the product of the linear ion density and the energy required to produce one ion-pair. It follows that the stopping power depends upon the energy of the α-particle in the same way as the linear ion density. If the stopping power is divided by the density of the medium, the *mass stopping power* is obtained; this is the energy loss of the particle after its passage through a layer in which behind a 1 cm^2 surface the unit mass of the medium is situated. In practice mainly the *relative stopping power* is used; this is generally defined as the stopping power related to air at 101 kPa and 15 °C. The

advantage of the relative stopping power is that it is practically independent of the particle energy.

α-radiation has a line spectrum, which indicates that a given radionuclide emits only α-particles of a given energy: the particles carry with them from the nucleus only discrete, possible energies. For instance, 93% of the α-particles of radium escape from the nucleus with an energy of 4.8 MeV, while 7% escape with an energy of 4.6 MeV. Evrey α-particle released from radon has an initial energy of 5.5 MeV. In accordance with the discrete energy values, the effective range too of these particles attains only definite values.

The ionization and excitation produced in a medium may induce various processes. Thus, atoms returning to their ground state emit *characteristic X-radiation.* Luminescent material (e.g. barium platinocyanide, or silver-activated zinc sulphide) produce visible light pulses (scintillations) on collision with α-particles. From a biological aspect the most important fact is that ionization and excitation may induce *chemical processes* resulting in *functional and morphological changes* of the tissues. The majority of the energy absorbed is finally transformed into *heat* in several steps.

There is also a small probability that an α-particle may interact with an atomic nucleus. If the energy of the α-particle is high enough, this interaction may lead to a *nuclear transformation.*

2.14.2. Beta-radiation (β-radiation)

1. β-decay. In the course of β-decay a *negative* electron or a *positive* electron *(positron)* is emitted from the nucleus. Electrons are not present in the nucleus, and accordingly β-decay requires some explanation. In processes within the nucleus one neutron may be transformed into one proton and one electron, or one proton may be transformed into one neutron and one positron. The nuclear symbols for the two kinds of β-particles are: ${}_{-1}^{0}\beta$, β^-, β and ${}_{+1}^{0}\beta$, β^+, respectively. Consequently, the transformations within a nucleus are

$$ {}_{0}^{1}n \rightarrow {}_{1}^{1}p + {}_{-1}^{0}\beta \quad \text{and} \quad {}_{1}^{1}p \rightarrow {}_{0}^{1}n + {}_{+1}^{0}\beta \qquad [2.40] $$

The symbol ${}_{0}^{1}n$ denotes the neutron, and ${}_{1}^{1}p$ the proton. The upper index is the mass number, and the lower index the charge. The former transformation is easy to interpret, since the mass of the neutron is somewhat greater than that of the proton (see also [2.45]) and can cover the mass of the electron produced. The transformation of a proton into a neutron may be explained in that part of the excess energy of the nucleus is devoted to ensuring the higher mass of the neutron (cf. [2.45]).

Negative β-decay is produced whenever more neutrons are in the nucleus than the number required to maintain stability; in positive β-decay the number of neutrons is smaller than required. In the case of negative β-decay the atomic number of the nucleus clearly increases by 1, whereas in the case of positive β-decay it decreases

by 1. The mass number remains the same in both cases. Negative β-decay is observed in both natural and artificial radioactive isotopes, but positron radiation is found only with artificial radioactive isotopes. As an example, the two radioactive isotopes of phosphorus may be mentioned:

$$^{32}_{15}\mathrm{P} \rightarrow {}^{32}_{16}\mathrm{S} + {}^{0}_{-1}\beta \quad \text{and} \quad {}^{30}_{15}\mathrm{P} \rightarrow {}^{30}_{14}\mathrm{Si} + {}^{0}_{+1}\beta \qquad [2.41]$$

2. Inverse β-decay. A nucleus with excess protons may decrease its positive charge not only by positron emission, but also by the capture of an electron from an inner shell, mainly from the K shell. As a result of the capture one proton is transformed into one neutron. This process results in the decrease of the atomic number of the nucleus by 1, while the mass number remains unchanged (similarly as in positron emission). This process is also called *shell electron capture,* or *K capture.* K capture occurs within the atom, and cannot be observed directly. However, when an electron jumps from one of the outer shells into the hole resulting from K capture, characteristic X-radiation is produced, which thus gives information about the K capture. The symbol of K capture is K.

3. β-radiation. The energy distribution of the β-radiation of an isotope is continuous: the energy of β-particles varies from zero up to the maximum value. However, it is surprising that the energy loss of a disintegrating nucleus is the same in all cases. This phenomenon is explained in that the β-particle is not emitted alone, but in the company of a neutral particle whose mass is a thousand times smaller than that of the electron; this particle is the *neutrino.* In the decay the two particles carry with them an identical overall energy, but this energy can be distributed between them in an infinite number of ways. If the electron acquires the total energy, a β-

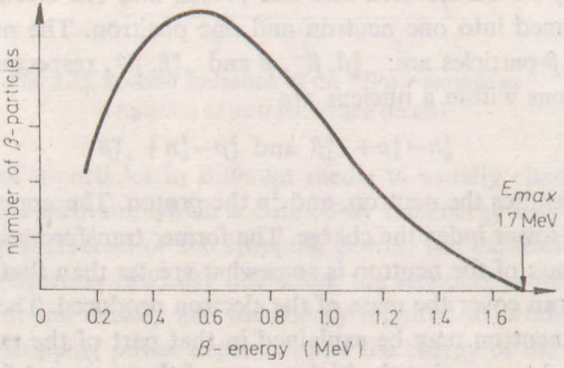

Fig. 2.34. The β-spectrum of $^{32}_{15}\mathrm{P}$

The abscissa gives the initial energy of the β-particles, and the ordinate the number of β-particles per unit energy interval. The maximum of the curve is at about 0.51 MeV. In the decay of $^{32}_{15}\mathrm{P}$, β-particles of this energy are produced with the highest probability. In the event of a symmetric curve, 0.51 MeV would be the average energy of the β-particles. Since the number of higher-energy particles is greater, the average energy is higher: 0.68 MeV

particle of maximum energy is ejected. The various isotope tables contain the maximum energy (E_{max}) of β-radiation. For instance, E_{max} is 1.7 MeV for the β-radiation of $^{32}_{15}P$. The energy spectrum of this isotope is depicted in Fig. 2.34. The lower-energy (softer) components of the β-radiation of an isotope obviously produce a smaller effect in any substance, or in the living organism, than higher-energy components. For an estimate of the expected effect, calculations are made only with an *average energy* (for $^{32}_{15}P$ this is 0.68 MeV), as if every electron had this same energy value.

The initial velocity of the β-particle approaches the velocity of light, and for this reason the relativistic mass increase must also be taken into account.

4. Interaction of β-radiation with matter. Because of the small mass of the electron and as a result of collisions and scattering, the track of a β-particle is rather zigzagged (in contrast to α-particles, β-particles are scattered by electrons). The degree of scattering may be even larger than 90° *(back-scattering)*. This circumstance must be considered in measuring technique as well as in radiation protection. Because of the continuous energy distribution, no uniform effective range exists; the effective range extends from 10 cm to several m in air, but is only a few mm in water and living tissues. The *specific ionizing power* of the β-particle is approximately 1000 times smaller than that of the α-particle. Naturally, the *stopping powers* of the various substances are smaller by the same order of magnitude for β-particles than for α-particles. Instead of the *stopping power*, the expression *linear energy transfer (LET)* is also used in the present case. For a velocity v the specific ionization in air is given by

$$s = \varkappa \left(\frac{c}{v}\right)^2 \qquad [2.42]$$

where $c = 3.0 \times 10^{10}$ cm s^{-1} and $\varkappa = 46$ ion-pairs/cm. In the case of high velocities ($v \approx c$), the specific ionization approaches the value of $\varkappa$, but for lower velocities s is larger than $\varkappa$.

Similarly to α-radiation, when β-radiation passes through various media it produces not only excitation and ionization, but also (as a consequence) chemical, photochemical, biological, etc. effects. *Characteristic X-radiation* too is generated, and (rather rarely) β-particles may suddenly be stopped in the field of an atom. When this occurs, *Bremsstrahlung* is produced.

An interesting phenomenon, *Cherenkov radiation*, should be mentioned here. Cherenkov radiation is observed if high-energy β-particles or other charged particles (accelerated electrons, protons, pions, etc.) interact with some medium. Cherenkov radiation is generated whenever a charged particle moves in some medium (e.g. water) with a velocity larger than that of light in the same medium. The primary process in this case too is the excitation of the atoms or molecules of the medium. If the velocity of the particle is lower than that of light, the light waves induced by excitation extinguish each other by interference. However, if the particle velocity is higher than that of light, the extinction is not total. The remaining bluish-white light is Cherenkov radiation. Similarly to other effects of the charged particle radiations this radiation too may be used to detect the (high-velocity) particles, to count them, to measure their velocity, etc.

The process leading to the attenuation (absorption) of β-particles is thus an extremely complex one. Nevertheless, it is interesting that in spite of this variety the absorption of β-radiation can be described within certain limits (section 2.3.1 and 2.10.1) by the equation

$$I = I_0 e^{-\mu x} \qquad [2.43]$$

For media consisting of elements of low atomic number, μ is linearly proportional to the density of the medium for a given radiation. Consequently, similarly as for X-radiation it is convenient to express the layer thickness not in cm or in mm, but in g cm^{-2} or mg cm^{-2}. In this way μ becomes independent of the nature of the substance, and depends only on the energy of radiation. Instead of μ, *the half-value thickness* D is frequently used in practice; this is given in units of g cm^{-2} or mg cm^{-2} instead of cm or mm. As an example, the half-value thickness of $^{32}_{15}$P β-radiation is approximately 110 mg cm^{-2}, which means that a 0.4 mm thick layer of aluminium with a density of 2.7 g cm^{-3}, or a 1.1 mm thick water layer, is required to reduce the intensity of radiation by half.

The above exponential law holds only for 3–4 half-value thicknesses; after this the intensity decreases more rapidly, and at 7–8 half-value thicknesses the intensity is suddenly extinguished *(maximum range)*. The maximum ranges of the β-radiation of some isotopes are presented in Fig. 2.35. To a first approximation the following equation holds between the maximum energy (E_{max}) and the maximum range (R_{max}) in the range 0.8–3.0 MeV:

$$R_{max} = aE_{max} - b \qquad [2.44]$$

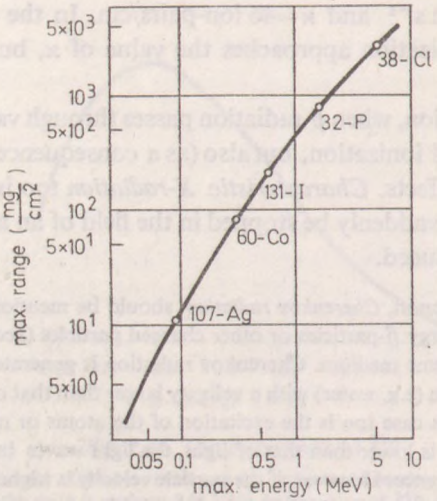

Fig. 2.35. Maximum range of β-radiation as a function of the maximum energy
Logarithmic scale on both axes

If E_{max} is expressed in MeV and R_{max} in mg cm^{-2}, the numerical value of a is 542, while b is 133.

There is no essential difference between negative and positive β-particles with regard to ionization, excitation, scattering and absorption. However, the life-time of a positron is extremely short. It interacts with a negative electron (with great probability towards the end of its track, when most of its kinetic energy has been lost), and on encounter their charges neutralize each other, and the two particles are annihilated as photons (γ-photons). The mass of the two interacting electrons corresponds to an energy of 1.02 MeV and this results in two annihilating γ-photons with 0.51 MeV energy propagating in opposite directions. In the case of positron radiation, particle annihilation always occurs, and consequently the presence of γ-radiation must always be taken into account in measurement technique and radiation protection.

Positrons are sometimes referred to as antielectrons, and the *positron–electron pair* is then called an *antiparticle pair*. Antipairs are pairs of particles whose mass, spin, magnetic moment, electric charge and other characteristic data are identical in absolute values, but opposite in sign. The antiparticles of the proton, the neutron and the neutrino are known: the antiproton, the antineutron and the antineutrino. (The photon is identical with its antiparticle.) It is generally true that when a particle collides with its antiparticle both are annihilated.

If only antiparticles are present they behave as common particles. Thus, atoms with a nucleus consisting of antiprotons and antineutrons and a shell containing positive electrons (antiatoms) are conceivable. Similarly to antiatoms, antimolecules and anticrystals may exist, in exactly the same way as common molecules or bodies are built up by common atoms. All of the macroscopic properties of antibodies would be the same as the properties of common bodies. Under the conditions on the Earth, there is no possibility for antibodies to exist. If an antiparticle is somehow created, within a very short time it encounters its corresponding particle and both are annihilated. In principle it is not impossible that antimatter does exist somewhere in the Universe, but there is no definite evidence of it.

It has already been mentioned that the continuous β-spectrum can be interpreted by assuming the existence of the *neutrino*. Since every β-decay is accompanied by the simultaneous appearance of a neutrino, all β-radiating isotopes are also strong neutrino-emitters. The neutrino may interact with a neutron or a proton, but the probability of this is extremely small. For instance, if 10^{12} neutrinos pass through the Earth, on average only one of them interacts with either a neutron or a proton. Thus the neutrino may cover a very large distance without transferring any energy to the medium through which it passes. For this reason their presence is neglected in medical isotope diagnostics and measurement techniques.

If the neutrino too is taken into consideration, the processes in [2.40] may be written in a more correct form:

$$n \rightarrow p + \beta^- + \bar{\nu}; \quad p \rightarrow n + \beta^+ + \nu \qquad [2.45]$$

It should be remembered that negative β-decay produces an antineutrino ($\bar{\nu}$), whereas positive β decay a common neutrino (ν).

2.14.3. Gamma-radiation (γ-radiation)

1. Prompt γ-radiation. After a nucleus has released a particle, it frequently still has some excess energy. In this case the resulting nucleus remains in an excited state. The excited nucleus releases its excess energy within a very short time (10^{-13}–10^{-18} s) in one or several steps by emitting γ-radiation. By this means the excited state is converted into a stable one. γ-radiation generally does not occur spontaneously; it is rather an effect accompanying some sort of corpuscular radiation. Independent γ-radiation is found only in rare isomeric transitions (see below).

γ-radiation is not accompanied by any change in atomic number or mass number. Let us consider as an example the decay of $^{198}_{79}$Au, which emits negative β-radiation with a half-life of 2.89 days, and a maximum β-energy of 0.96 MeV. The $^{198}_{80}$Hg nucleus formed immediately after the β-decay still possesses an excess energy of 0.41 MeV, which is released by the ejection of a single γ-photon. After this the nuclear derivative is stable. The γ-radiation is emitted by the excited nuclear derivative and not by the original isotope, yet in practical terms the prompt γ-radiation is ascribed to the primary nucleus, and $^{198}_{79}$Au is said to be an isotope emitting β- and γ-radiation. Isotopes which emit γ-radiation besides some particle are *mixed-radiating* isotopes, in contrast to the *purely* α- *or* β-*radiating* ones.

2. Isomeric transition. Some radioactive nuclei do not radiate their excess energy as γ-photons immediately after the particle emission, and the nuclear derivative remains for a relatively long time ($>10^{-10}$ s) in an excited state, returning to the ground state only with a definite half-life. Such a transformation takes place e.g. in the following process:

$$^{99}_{42}\mathrm{Mo} \xrightarrow[\beta-]{66\ \text{hours}} {}^{99\mathrm{m}}_{43}\mathrm{Tc} \xrightarrow[\gamma]{6\ \text{hours}} {}^{99}_{43}\mathrm{Tc}$$

Above the arrows the half-lifes, below them the type of decay are indicated. The $^{99\mathrm{m}}_{43}$Tc isotope, an intermediate product of the reaction, is transformed into a stable technetium isotope with a half-life of 6 hours by γ-emission. Thus technetium nuclei may exist simultaneously in excited and stable state for a well measurable time. The excited nucleus is called an *isomer* of the nucleus in the ground state and the phenomenon is *nuclear isomerism*. The isomeric nucleus is denoted by the letter m beside the mass number (metamorphose).

The $^{99\mathrm{m}}_{43}$Tc isotope is used in medical practice, since its properties are advantageous in several respects (cf. section 2.18.3). Here it only has to be noted that the life-time of $^{99\mathrm{m}}_{43}$Tc nuclei is long enough to ensure the practically usable quantity of active technetium nuclei in a technetium preparation separated chemically from molybdenum. Thus, one has a method to produce a substance which emits only γ-radiation. With the above outlined *technetium generator* a new active isotope may be obtained at the site of applications daily several times. Cases are known when the radioactive decay of an excited nucleus follows the primary emission of γ-photons.

3. Interaction of γ-radiation with matter. The nature of γ-radiation is similar to that of X-radiation, and thus their effects are essentially the same (cf. section 2.10).

Mention may be made of the *internal photoeffect*. With certain atom types the γ-photon emitted by an excited nucleus may produce photoeffect in the *same atom*, i.e. the excitation energy is captured by a shell electron which can then escape from the atom. In these cases the escaping electron, called a *conversion electron* (and the X-radiation produced) is observed instead of γ-radiation. The conversion electron is generally ejected from the K shell; its symbol in nuclear reactions is e^-.

The data discussed in connection with the attenuation (absorption) of X-radiation in section 2.10 also relate to γ-radiation. Some additional findings may also be dealt with. Figure 2.36 depicts the dependence of the mass attenuation (absorption) constant for water on the photon energy (or the wavelength). Conclusions can be drawn from the curves about the absorption by the soft tissues. From a practical aspect the following data are of interest: the half-value thickness of γ-radiation of Ra in lead is 1.3 cm, that of $^{24}_{11}$Na is 3.5 cm, and that of $^{131}_{53}$I is approximately 0.4 cm. Depending upon the wavelength, the half-value thickness of air is several hundred m, and that of the living organism several dm.

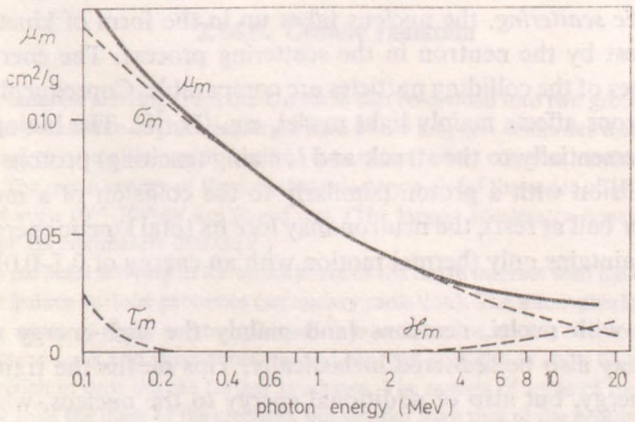

Fig. 2.36. Total and partial attenuation coefficients of water for X-radiation and γ-radiation

2.14.4. Neutron and proton radiation

1. Neutron radiation is produced by certain nuclear processes, mainly by bombardment of the nucleus with appropriate particles (including photons). The resulting highly-excited nucleus emits a neutron. The capture of the particle and ejection of a neutron occur within a very short time (10^{-15}–10^{-18} s). (Only a small number of nuclear processes are known in which the neutron emission occurs with a measurable half-life.) As an example, the bombardment of $^{9}_{4}$Be with γ-photons may be mentioned.

Following γ-energy absorption, the nucleus emits one neutron and is transformed into the ^{8}Be nucleus:

$$^{9}_{4}\mathrm{Be} + h\nu \rightarrow {}^{8}_{4}\mathrm{Be} + {}^{1}_{0}n$$

or in brief:

$$^{9}_{4}\mathrm{Be}(\gamma,n)^{8}_{4}\mathrm{Be}$$

The first symbol in the bracket indicates the bombarding species entering into the nucleus, while the second refers to the ejected particle.

The *free neutron* is an unstable product, with a half-life of approximately 13 minutes; it disintegrates into a proton and an electron.

Since the neutron has no charge, it *does not cause direct ionization.* On passing through some medium it interacts only with the nuclei, and not with the electrons. In this respect, two processes are of interest: *neutron scattering,* and the *production of nuclear reactions.*

Neutrons are generally ejected from the site of their generation with high energy (a few MeV). During their progress, however, they transfer part of their energy by scattering to the atomic nuclei, and continue travelling in a changed direction. In the case of *elastic scattering,* the nucleus takes up in the form of kinetic energy the kinetic energy lost by the neutron in the scattering process. The energy transfer is larger if the masses of the colliding particles are comparable. Consequently, the energy loss of fast neutrons affects mainly light nuclei, e.g. $^{1}_{1}\mathrm{H}$, $^{2}_{1}\mathrm{H}$. The biological effect of neutrons is due essentially to the struck and ionizing (exciting) protons. In the event of a central collision with a proton (similarly to the collision of a moving billiard ball with another ball at rest), the neutron may lose its total kinetic energy in one step, after which it maintains only thermal motion with an energy of 0.1–0.01 eV *(thermal neutron).*

On colliding with nuclei, neutrons (and mainly the high-energy neutrons, the fast neutrons) may also be scattered inelastically. This means the transfer not only of the kinetic energy, but also of additional energy to the nucleus, which results in the promotion of the nucleus to a higher energy level. The nucleus becomes excited, and releases its excess energy by emitting one or more γ-photons. Thus, the inelastic scattering of neutrons is accompanied by γ-radiation emitted by the excited nuclei.

The other interaction between nuclei and neutrons is manifested in the production of nuclear reactions. The neutron has proved to be an extremely suitable bombarding particle in transforming nuclei, since it is electrically neutral, and easily penetrates into the nuclear force field. The neutron is captured by the nucleus, which generally ejects one proton or one γ-photon. More rarely, after capturing a fast neutron, the nucleus may eject another neutron, or possibly even two. Neutron capture is sometimes followed by the ejection of an α-particle.

Both scattering and nuclear reactions result in the attenuation of neutron radiation. Considering the low probability with which a nucleus captures a fast neutron

as compared to a slow (thermal) neutron, in practice fast neutrons become decelerated in a series of elastic and inelastic scattering steps, and the resulting slow neutron finally produces the nuclear reaction. The attenuation of neutron radiation is described by the well-known equation

$$I = I_0 e^{-\mu x} \qquad [2.46]$$

Here too the attenuation constant depends on the nature of the absorbing medium and on the energy of the neutrons.

2. Proton radiation is produced either by the acceleration of hydrogen ions or, (similarly to neutron radiation) by bombardment of the nucleus with some particle (including photons). In the latter case the capture of the missile and the ejection of the proton generally follow each other within a very short time. Like all other particles bearing an electric charge, the proton causes ionization and excitation as it passes through a medium. The linear ion density is smaller than for α-particles, but larger than for electrons.

2.14.5. Cosmic radiation

The *cosmic radiation* arriving from the Universe can be divided into two groups. One group consists of the *primary radiation* at altitudes larger than 25–30 km, and comprises mainly (approximately 91%) protons and to a smaller extent (ca. 8%) α-particles; more complex nuclei (up to nickel) also occur in traces. The mean energy of these particles is very high (of the order of 10 GeV), but particles with energies of even 10^{14}–10^{19} eV are found too. (The largest accelerator constructed to date produces protons of approximately 300 GeV.)

The primary particles arriving in the atmosphere of the Earth interact with the atmospheric atoms, and in doing so induce various processes (secondary radiation). The atmospheric atomic nuclei may undergo fission, some of them are transformed into radioactive nuclei, neutrons are emitted, and γ-radiation, electron pairs and various neutral and charged particles of short lifetimes (10^{-16}–10^{-6} s) are produced, which in turn initiate further processes. The masses of some of the short-lifetime particles are greater than the mass of the electron, but smaller than that of the proton (mesons), whereas the masses of others lie between the mass of the proton and that of the deuteron (these are the hyperons).

The secondary cosmic radiation consists of soft (easily absorbed) components and hard components with a considerable penetrating power. The soft components are mostly electrons and photons, and the harder ones are mainly mesons. For instance, more than 10 mesons per second pass through the human body. The soft components are absorbed by a lead layer a few cm thick, whereas the hard components easily pass through a 1.0 m thick lead wall.

It is currently assumed that *cosmic radiation originates in the supernovae*, stars of varying brightness. These suddenly increase enormously in brightness, while their internal temperature attains a value of 10^9 K. The high-energy protons and other particles emitted race throughout the Universe for millions of years before reaching some celestial body, e.g. the Earth.

2.14.6. Decay schemes of radioactive isotopes

In order to be able to measure and utilize the radioactive isotopes, it is essential to know the details of their decay. Figure 2.37 presents a few decay schemes which are characteristic of isotopes of interest in medical practice and research. The nuclei have *discrete energy levels*, indicated by horizontal straight lines; the higher lines represent higher energy levels. An increase in atomic number (negative β-decay) is denoted by a displacement to the left, and a decrease in atomic number (positron decay, K capture) by a displacement to the right. The physical half-life is given in brackets beside the symbol of the primary nucleus. Diagram *a* depicts the decay scheme of the purely β^--decaying $^{32}_{15}P$, and diagram *b* that of the purely β^+-decaying $^{11}_{6}C$. $^{198}_{79}Au$ is a mixed radiating isotope, which

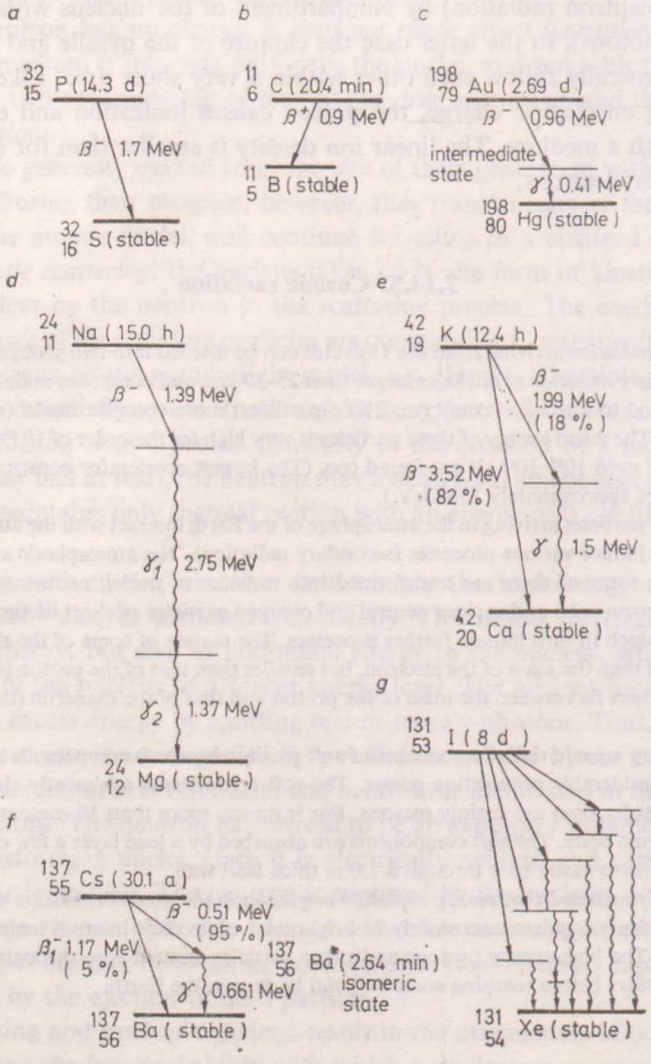

Fig. 2.37. Decay schemes

first undergoes negative β-decay and then emits a 0.41 MeV γ-photon. With $^{24}_{11}$Na the remaining excitation energy is released as not one but two γ-photons. 82% of the $^{42}_{19}$K nuclei are purely β-radiating and 18% mixed radiating isotopes. (It is worthwhile remembering the definition of activity, which is based on the number of atoms disintegrating per unit time. It should be added that this definition holds quite independently of the decay scheme. If, for instance, 10^7 nuclei disintegrate per second in a $^{24}_{11}$Na preparation, this means 10^7 β-particles, but twice as many γ-photons. With a $^{42}_{19}$K preparation of the same activity, on the other hand, when the number of β-particles is 10^7 per second only about one-fifth as many γ-photons should be considered in the calculations. This fact must always be taken into account before any experiment or medical examination is carried out.) Figure 2.37*f* depicts the $^{137}_{56}$Ba isomeric transition which follows $^{137}_{55}$Cs β-decay. Finally, diagram *g* presents the rather complex decay scheme of $^{131}_{53}$I. In this latter scheme the numerical data have not been given. It need only be mentioned that $^{131}_{53}$I emits β^--particles with a mean energy of 0.2 MeV together with mainly 0.36, 0.08 and 0.72 MeV γ-photons.

2.14.7. Particle accelerators in medicine

Charged particles (electrons, protons, deuterons, helium ions and other ions) are accelerated in an electric field. The high-energy particles obtained are utilized in various ways in both medical practice and research. In recent years different types of accelerators have been developed, mainly for use in nuclear physics. Of these, however, only the basic types which are of interest in medicine will be described.

1. The betatron is the most frequently used electron accelerator; it can also be employed to produce hard X-radiation (cf. section 2.9).

In the betatron the electrons are accelerated by an alternating magnetic field which acts in two different ways, partly by spinning the injected electrons in a circular orbit, and partly by accelerating the electrons in the electric field it induces (*electromagnetic induction,* Fig. 2.38*a*). The equipment (Fig. 2.38*b*) consists of a suitably shaped electromagnet at the centre of which a ring-shaped vacuum tube is situated. The electrons to be accelerated are admitted to the tube via an injector. A 50 Hz alternating electric current induces an alternating magnetic field, which in turn creates an electric field in the vacuum ring. However, the electrons can be accelerated only with an electric field acting in a given direction. In the betatron, approximately a quarter period is used for acceleration. Within this time about 100,000 rotations are completed. After this, the accelerated electrons are diverted from their orbit by an appropriate electric field in order to utilize them for the desired purpose. By means of the betatron, electrons with a maximum energy of several hundred MeV are obtained.

2. In the cyclotron the particles (mainly ions) are accelerated by a voltage of several hundred thousand volts, but they can traverse the accelerating space approximately 200 times and they accumulate excess energy with every turn.

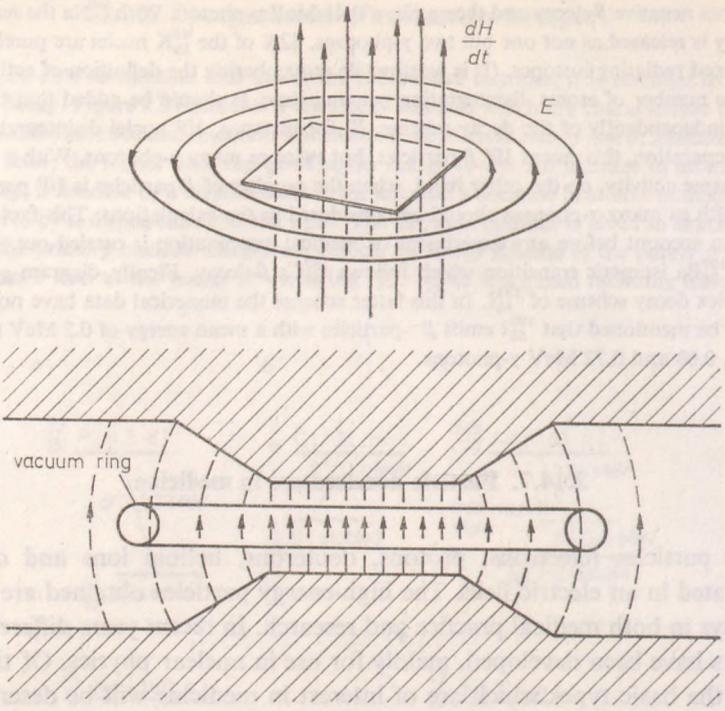

Fig. 2.38. Outline of operation of the betatron
a: the variation of the magnetic field in time *(dH/dt)* produces an electric field *(E)*;
b: betatron cross-section. The dashed lines are the magnetic lines of force

An essential part of the equipment is a flat, cylindrical metal box (with a diameter of several m), which is cut in two halves along its diameter (Fig. 2.39). The accelerating voltage is applied to the two parts of the box, called dees (*D*-electrodes). An electric field is produced practically only in the slit between the electrodes, the field intensity within the boxes being zero. The ions to be accelerated are produced by the ion source protruding into the centre of the box. Within this box the ions move with increasing velocity along a spiral path. The acceleration is provided whenever the ions pass through the slit. This occurs twice per rotation, in opposite directions. In order to induce an acceleration in every period, an alternating field is required. Consequently, an alternating voltage is applied to the dees. The curvature of the path is ensured by a magnetic field, whose lines of force are perpendicular to the plane of the box. With increasing velocity the radius of the orbital path increases too; this is the reason for the spiral particle orbit. The accelerated ions are deviated from their orbit after passing the electrode *K*, escape through the window *W* and hit the target.

With the first cyclotrons, protons could be accelerated to an energy of 20 MeV. In the variants developed later, such as the synchrocyclotron, the synchrophasotron, etc., protons with an energy several orders of magnitude higher (up to a few GeV) may be obtained. For medical purposes, mainly small and medium cyclotrons (up to approximately 50 MeV) are used.

The importance of these cyclotrons lies in the fact that the accelerated particles can be employed to initiate a large number of nuclear reactions providing radio-

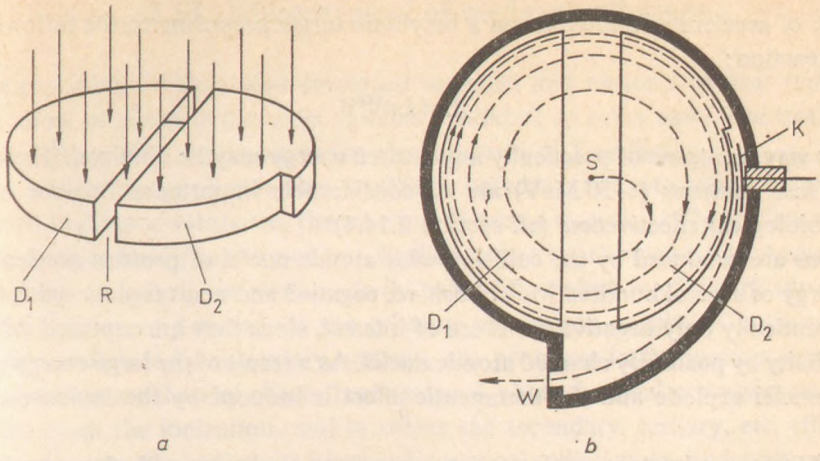

Fig. 2.39. Principle of operation of the cyclotron

a: the dees (D_1 and D_2) and the magnetic lines of force; *R* is the slit between the dees; *b:* the dees viewed from above with the deflecting electrode *K;* the helical line is the path of the ions starting from the source *S*; *W* is the exit window

isotopes with nuclear parameters favourable for tracing purposes (for use in both research and diagnostics, cf. section 2.18). The use of *isotopes with shorter half-life* (^{123}I, ^{11}C, ^{81}Rb, etc.) is advantageous in medical practice, since with these the dose exposure is smaller (cf. point 3 of section 2.18), several examinations can be carried out in a short time, the medical check-up is simplified, and so on.

On-site isotope production is especially favourable with elements possessing only isotopes with very short half-lives, for instance oxygen and nitrogen, which are important in some functional examinations (the isotope parameters are listed in Table 8.14).

These ideas are obviously also fundamental in scientific research.

In connection with the medical applications of cyclotrons, it should be emphasized that some of the short half-life isotopes produced by these accelerators emit only γ-rays, which are obviously more favourable from the viewpoint of dose exposure than those isotopes of the same element which emit mixed radiation. The possibility of producing short half-life positron-emitting isotopes should also be borne in mind. These isotopes allow very exact localization in certain diagnostic examinations.

The cyclotrons (and their developed variants) may become an important tool in therapy. As a result of recent investigations it is expected that besides X-radiation, γ-radiation and accelerated electrons (not to mention α- and β-radiation), high-energy protons and heavier charged particles (atomic nuclei, fission products), neutrons and pions (cf. section 1.1) will also be of therapeutic use.

Appropriately accelerated *protons and heavier particles* are obtained directly from the accelerator. *Neutrons,* on the other hand, are usually produced by the

impact of accelerated deuterons on a beryllium target according to the following nuclear reaction:

$$^{9}_{4}\mathrm{Be}(d,n)^{10}_{5}\mathrm{B}$$

In this way, neutrons of practically any desired energy may be obtained. Therapeutically, fast neutrons (>30 MeV) are of considerable importance because of their great biological effectiveness (cf. section 2.14.4).

Pions are produced by the collision with atomic nuclei of protons accelerated to an energy of several hundred MeV. Positive, negative and neutral pions exist, though therapeutically only negative pions are of interest, since they are captured with high probability by positively charged atomic nuclei. As a result of the large energy uptake, these nuclei explode and the therapeutic effect is induced by the fission products.

3. Linear accelerators accelerate ions as well as electrons; with these equipments it became possible to produce electrons of many GeV, and protons of a few GeV. (The accelerated electrons may be used subsequently to produce hard X-rays.) Differently from cyclotrons, here the particles are accelerated on a linear path, hence the name: linear accelerator. Their advantage, as opposed to accelerators of other type, is the production of beams of relatively large intensity, and homogeneous irradiation fields which is favourable concerning their therapeutical application.

Figure 2.40 demonstrates an arrangement in which the path leads within a sequence of coaxially placed, tube-like electrodes working with an alternating current of properly selected high frequency. In these equipments — similarly to cyclotrons — electric field is produced only in the gaps between the electrodes, within the cylinders the electric field is zero. The particles are accelerated only when passing through the gaps. The motion of the particles as well as the length of the cylinders and the frequency of the alternating current must be co-ordinated so that the most favourable part of the period of the alternating field (concerning direction and field strength) should affect the particles passing through the gaps (resonance method.) By increasing the velocity the path length of the particles will be increasingly longer, which must be taken into account by increasing the length of the electrodes, which is shown in the figure.

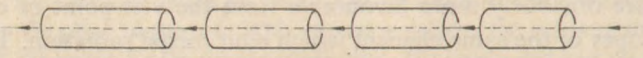

Fig. 2.40. Diagram of a linear resonance-accelerator with cylindric electrodes. The dashed line with arrows denotes the path of the particles

Every accelerator belongs to the so-called large equipments, whose operation and maintenance require a specially trained technical personnel and specially constructed laboratories.

2.15. Measurement of nuclear radiations

Various methods have been developed to detect and measure nuclear radiations. These allow selection of the most suitable procedure in every case. The methods to be outlined are also applicable to the measurement of X-radiation.

The measurement of radiation is always based on the interaction between the radiation and some substance, the medium (detector material). The radiation transfers some of its energy to the medium. Radiation passing through without energy loss does not leave a trace in the medium. In the case of the radiation of electrically charged particles (e.g. α- or β-radiation) the primary phenomena consist of ionization and excitation, followed by secondary, tertiary, etc. processes in the various substances. Such processes are thermal effects, luminescence, photochemical processes, and so on. In some cases the ionization, and in others the secondary, tertiary, etc. effects are used to measure the radiation. Electrically neutral radiation (γ- and neutron radiation) first produces electrically charged particles in the detector, and these subsequently display the effects already discussed. Thus, in the measurement of γ-radiation the photoelectrons, Compton electrons and electron–positron pairs are utilized, while in the case of neutron radiation the neutron-struck protons or the charged particles (e.g. α-particles) produced by neutron-induced nuclear reactions are used. Radiation consisting of charged particles is frequently referred to as *directly ionizing*, whereas radiation of neutral particles is *indirectly ionizing* radiation.

Of the large variety of types of nuclear radiation, γ- and β-radiation are used most frequently in medical practice and in biological investigations. Accordingly, stress will be laid on the measurement of these.

1. Measuring devices based on gas ionization. One large group of measuring instruments is that of *ionization chambers, proportional counters,* and *Geiger–Müller tubes*. In this family of instruments charged particles move in an electric field and collide with gas molecules, which become ionized. The ions or ion-pairs produced are accelerated by an electric field in the tube or chamber. Depending on the sign of their charge, the ionized particles move to the positive or negative electrode of the apparatus (Fig. 2.41). The measurement of the radiation is based on measurement of the ionization current. In ionization chambers the total ionization produced by a large number of ionizing particles is generally measured, whereas the Geiger–Müller counter (abbreviated to GM counter) is used to count the number of current or voltage pulses (particle counting). The proportional counter allows not only counting of the particles, but also distinction between them according to their type and energy.

An understanding of the different processes occurring in *gas-filled chambers* is facilitated by Fig. 2.42. The diagram relates to cylindrical chambers whose negative electrode is the cylinder wall, while the positive electrode is an axially mounted metal wire. The abscissa indicates the voltage between the cylinder and the axial wire, and the ordinate gives the number of ions (electrons) participating

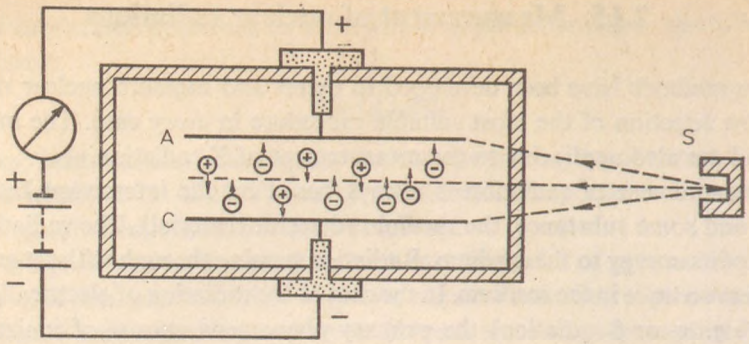

Fig. 2.41. Outline of operation of an ionization chamber

The small circles between the electrodes (*A* and *C*) denote the charge carriers produced by radiation. *S* is the source (e.g. γ-source)

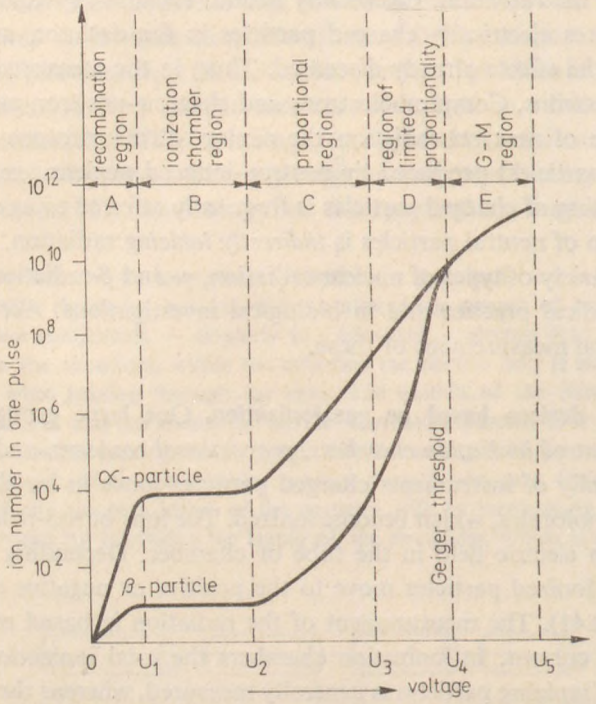

Fig. 2.42. Pulse amplitude of counters as functions of voltage

in the pulse produced by one particle in a given case. The upper curve is for α-particles, and the lower one for β-particles. With a small voltage (range *A*) some of the ions produced by the particles recombine before arriving at the respective electrode (recombination range). On increase of the voltage, every ion produced by the particle arrives at the electrodes, and thus the saturation current is measured. The ionization chambers operate in this voltage range (range *B*). (Because of its higher ionization potential, the α-particle produces more ion-pairs than the β-particle.) At even higher voltages, as a result of the induced ionization the electric pulses on the wire increase by several orders of magnitude,

and an amplification (internal or gas amplification) takes place within the tube, but the number of pulses is still proportional to the number of primary ion-pairs produced by the particle (range *C*). The proportional counter operates in this range. On further increase of the voltage across the tube, the restricted proportional range (range *D*) is attained, where the proportionality between the primary ionization and the amplified pulse is gradually lost. At a sufficiently high voltage the difference between the pulses due to the particles of various types and energies disappears, the pulses become uniform. This voltage, which depends strongly on the size and structure of the tube, and also on the pressure and nature of the filling gas, is the *Geiger threshold.* In counting tubes operating in the Geiger–Müller range the internal amplification may be higher than 10^8; this results in pulses which are large enough to be further amplified electronically in a simple and reliable way (cf. section 5.3). This is the advantage of the GM counters. On further increase of the voltage, self-maintained discharges occur, which result in the rapid destruction of the tube.

Ionization chambers are mainly used at present in individual radiation protection. They are easily manageable devices (e.g. pocket dosimeters of the size and shape of a fountain pen can be made); they can be fastened on the clothing, which permits easy control of the dose obtained during work by personnel handling radiating substances. In principle these chambers are charged electrometers, which progressively become discharged in response to the radiation. The dosimeters for calibration purposes are ionization chambers, and thimble chambers too are frequently used in radiological practice (cf. section 2.17.2).

GM tubes are mainly used to detect β-particles. Tubes made of some suitable material and filled with some appropriate gas are also applied to count neutrons. For γ- and X-ray photons, especially high-energy ones, the efficiency of GM tubes is only 0.1%, and for this reason they are used only exceptionally in these cases.

2. Detectors based on luminescence. These detectors are usually made of inorganic or organic crystalline materials, but fluid luminophores are also used. Every charged particle striking the detector produces a scintillation (light pulse). The total light emitted is usually measured, and from the intensity of light the intensity of the nuclear radiation is inferred. Another method of application is to count the individual light pulses and hence to determine the activity of the radioactive sample. In either light intensity measurements or the scintillation counting method, the light is first converted into an electric signal by a photomultiplier; this signal is then amplified and processed (cf. section 5.6.2).

The measurement of γ-radiation has been seen to be frequently applied in medical practice. For this purpose mainly scintillation counters are used; thallium-doped (activated) NaI crystals a few cm in size are generally built in as detectors. By means of well-known effects (cf. section 2.10.2), on striking the large density (approximately 2.5 g cm^{-3}) NaI crystals containing the high atomic number iodine component, it is highly probable that the γ-photons lose some or all of their energy by absorption, and consequently the efficiency of the photon counting is very good (it may amount to 60–80%). In fact, it is several orders of magnitude better than the γ-efficiency of GM counters. In clinical practice, where the quantity of radioisotopes used in hu-

man diagnostics should be as low as possible, the large γ-efficiency is not only advantageous, but is considered to be a requirement for protection. The unit consisting of the scintillation detector, the photomultiplier and the first electronic amplifier is the *scintillation head.* Figure 2.43 outlines the construction of the detector and the photomultiplier, while Fig. 2.44 (in the Supplement) illustrates the complete counting device.

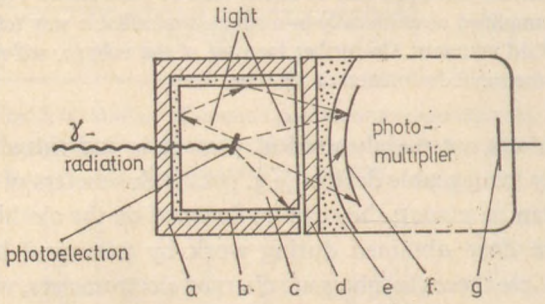

Fig. 2.43. Outline of scintillation counter for γ-counting
a: light-proof case; *b:* light-reflecting layer; *c:* NaI(Tl) crystal; *d:* glass plate; *e:* plexi disc (light guide); *f:* photocathode; *g:* anode

A particle stopped in the scintillation detector (for instance a photoelectron produced by a γ-photon) interacts several times with the detector until its total energy is lost. As a result, one particle creates several luminescent photons. The average number of these photons is proportional to the energy of the interacting particle; this greatly enhances the applicability of luminescence detectors, since from the magnitude of the light pulse produced and after conversion the magnitude of the electric pulse, the energy of the particle can be determined. If the particle is, for instance, a photoelectron produced by a γ-photon, the energy of this γ-photon can be determined from the generated electric pulses. The scintillation technique allows the determination of the γ-spectrum of the radioactive preparation, and hence the identification, control and determination of its composition.

β-particles are detected with anthracene crystals or detectors made of plastic (plastic phosphors). Fast neutrons are usually detected with some plastic substance rich in hydrogen; slow neutrons are measured with substances containing boron, and α-particles are most suitably detected with thin layers of zinc sulphide doped with silver.

The measurement of β-particles of very low energies (e.g. the maximum of the β-particle energy of tritium is only 0.0183 MeV) is most conveniently carried out with *liquid scintillators.* In this method the sample to be measured is mixed with the scintillator fluid, and the mixture is placed on the window of a photomultiplier.

High-energy charged particles are also measured by means of *Cherenkov radiation (Cherenkov counters).* As a medium, generally distilled water is used and the light signals are processed in the same way as in the other methods.

3. Photochemical measuring devices. The effects produced on photographic plates are frequently used to indicate and measure radioactive radiation. This method is suitable for study of the total emission, i.e. the *total amount of radiation,* and also the *individual particles.* In the former case the darkening of the emulsion due to radiation is determined by means of a photometer (densitometer), and hence the energy falling on unit surface of the emulsion is deduced. In the latter case the tracks of the individual ionizing particles in the emulsion are studied microscopically, and as a result the type, energy, etc. of the particle are determined.

The *photoemulsion method* in many instances allows a relatively exact determination of the location and distribution of the radioactive substances. This may provide valuable information in biological and histologic studies. While measurements with the GM counter or the scintillation technique yield information of the total isotope content of some larger size tissue or organ, the latter method, *autoradiography,* permits the localization of the isotope within a given organ, cell cluster, or even individual cells (see Fig. 2.45, in the Supplement). In a frequently used procedure 5–20 μm thick sections are made from the biological object to be examined and are then contacted with a photosensitive emulsion (e.g. by smearing a 1–2 μm thick emulsion on the section). After a suitable exposure time and subsequent development, depending upon the concentration of the isotope, dark spots of different sizes are observed. The autoradiogram provides the more information, the better it reflects the distribution of the isotope in the histologic section. With the most advanced methods, even spots only 1–2 μm apart from each other can be distinguished.

Besides the ionization of gases, luminescence and photochemical effects produced in certain substances, other effects of ionizing radiation are also used for detection and measurement, e.g. *thermal* and *chemical effects,* and changes produced in the *electric conductivity, absorption spectra* or other optical properties of solids (cf. section 2.17.3).

2.16. Dosimetry. Basic concepts

This section will deal with those problems of radiation measurement which are important because of biological effects.

Ionizing radiation initiates in the human body destructive processes. However, the damage is usually not manifested at once, but only after some time, possibly years or even decades later. Consequently, quantities must be found which permit characterization of the various types of radiation from the aspect of their expected biological effects, so that these may be inferred in advance. Such problems are dealt with in *dosimetry.*

The concept of dose has been taken over from pharmacology, where it means the drug quantity administered into the organism in various ways. More exactly, the term dose denotes the administered drug quantity per weight or mass unit. The expression *radiation dose* refers quite generally to biologically effective quantities taken up by the organism and related to mass (or volume) units. There is a consider-

able difference between the two concepts of the dose. In pharmacology the total drug quantity administered is considered, regardless of the effective quantity taken up by the organism, and also regardless of the quantity taken up, but secreted by the organism without exerting any effect. With radiation, however, the situation is different, since in radiodosimetry only the effective quantity is considered, i.e. only the energy absorbed by the body or organism. Radiation passing through the organism without any interaction is not taken into consideration. After these preliminaries the basic objective of radiodosimetry may be formulated as *the determination of the energy absorbed by the tissues in a given region.*

It is important to emphasize that the absorbing region, which may be located in various depths, may contain different tissues. Because of the scattered radiation, surroundings of these regions are also considered. The dose must be known from area to area and almost from point to point, and the measurements are carried out only where this is actually necessary and justified by the circumstances. It should also be taken into consideration that, though the knowledge of the absorption of biologically effective radiation is of basic importance, it is in itself not sufficient, because other physical, chemical and biological factors are also important in the development of the biological effects.

It clearly follows that the measurement of *radiodoses* requires *special* efforts, and the solution of the problems involves new concepts and measuring techniques. Many problems have still not been satisfactorily solved, and a more exact knowledge of the physical, chemical and biological effects will probably lead to further development in this field.

2.16.1. Physical dose concepts

1. Absorbed dose. Denote the energy absorbed by some mass $dm\,(=\varrho dV)$ of the body by dE. The absorbed dose in this region is given by

$$D = \frac{dE}{dm} = \frac{1}{\varrho}\frac{dE}{dV} \qquad [2.47]$$

The numerical value of D is equal to the energy absorbed by unit mass; its unit is $J\,kg^{-1}$, i.e. the *gray* (denoted by Gy).

The former unit was the rad (radiation absorbed dose):

$$1\ \text{rad} = 0.01\ \text{Gy} \qquad [2.48]$$

2. Exposure. While the absorbed dose is valid for any kind of absorbed radiation, the exposure refers only to *X- and γ-radiation.* An even more essential difference between the two types of doses is that, whereas the former provides direct information on the energy absorbed, the latter characterizes only the *ionizing power* of the radiation in the air, which consequently gives only indirect information about the

radiation actually absorbed by the tissue. If the exposure in some region of the tissues is mentioned, this actually refers to the ionization produced by the X-rays or γ-rays in the air rather than in the tissues. From a practical medical aspect this is sufficient in many cases, especially if it is considered that the measurement of exposure is relatively simple. Thus, it is understandable that even to-day this is often preferred to the more general *absorbed dose* which provides more exact information and has been employed for more than 50 years.

Before the exposure is defined, some considerations are required, since ionization measurements in the air may be carried out under different conditions. The definitions should also contain the conditions of measurement.

Figures 2.46 and 2.47 depict two extreme cases. A common feature of the two cases is the air-filled cavity separated by a wall from the surrounding tissues. In both cases the free positive and negative charges produced by the ionization of the air in the cavity are measured, though the number of charges produced in the air is influenced in different ways by the environment. The role of the surroundings may be understood if the processes induced by the radiation are considered in detail.

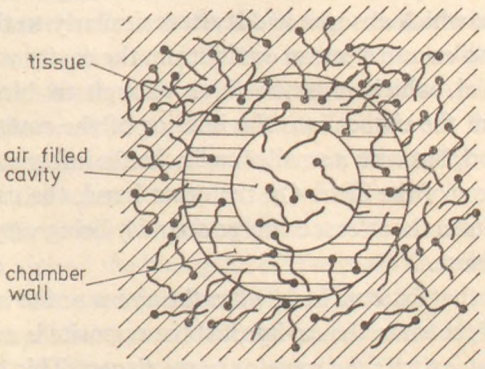

Fig. 2.46. Diagram relating to the measuring chamber operating on the principle of electron equilibrium

Not only in the air, but also in the tissues and in the wall surrounding the cavity does radiation induce photoelectrons, Compton electrons, and possibly electron–positron pairs (referred to below as *secondary electrons*), which cover various distances before losing their energy by ionization (and excitation). In the diagrams the points indicate the sites where the electrons are produced, and the irregular lines denote the tracks of the secondary electrons. It should be emphasized once more that only charges produced by secondary electrons in the air are measured. These secondary electrons may possibly travel only a short distance in the air, and cross the wall before being stopped. Obviously, the charges produced by the secondary electrons in the remaining section of their track will not be measured. However, some secondary electrons produced in the wall or (if the wall is thin) in the surrounding tissue may

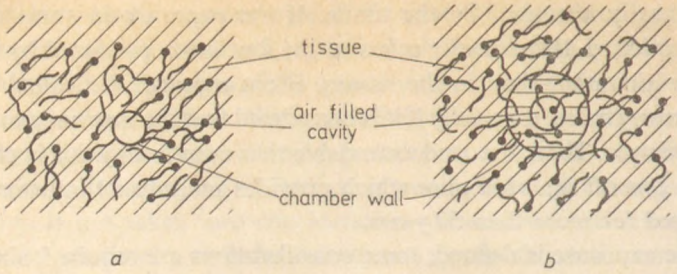

Fig. 2.47. Diagram relating to the measuring chamber operating on the Bragg–Gray principle

complete their track in the air, and the charges they produce in the air will be measured. The material (and the thickness of the wall) can be chosen so that the number of charges lost due to the secondary electrons leaving the cavity will be balanced by the charges due to electrons emitted from the surroundings of the cavity into the air. In this case the charge density within the cavity will be the same as if the air in the cavity were surrounded by air. This leads to electron equilibrium, depicted in Fig. 2.46. Every substance which absorbs and scatters similarly to the air is said to be air equivalent. In order to ensure electron equilibrium the cavity walls are made of some air-equivalent material, which must be thick enough to block the access of the electrons ejected from the tissues into the interior of the cavity. The dimensions of the cavity are not fixed, but with a smaller cavity better information is obtained about the spatial distribution of the dose. On the other hand, the cavity must not be too small, since this results in the released charge quantity being very low and consequently impossible to measure.

Figure 2.47 shows that extreme case, when the aim is not to attain electron equilibrium characteristic of the air in the cavity. Just the opposite is achieved: *the secondary electron density in the cavity is the same as in the tissues.* This happens whenever the dimensions of the cavity are small compared to the effective range of the electrons in the tissues. The cavity wall is extremely thin in this case (Fig. 2.47*a*), so that the electrons can pass through with practically no attenuation.

Another solution (Fig. 2.47*b*) is for the wall to be made of some material which behaves similarly to the tissues, but not to the air, i.e. the wall is made of some *tissue-equivalent substance.* The procedure outlined in Fig. 2.47*a*, *b* is the *Bragg–Gray method.*

Both solutions provide the possibility of dosimetry, i.e. one can measure on the basis of electron equilibrium, or according to the Bragg–Gray method. The former one was actually the first to be achieved, and whenever exposure is mentioned this method is thought of.

After these preliminaries the exposure can be defined. Let dq denote the positive or negative charge produced at *electron equilibrium* by ionization in air of mass dm and volume dV ($dm = \varrho\, dV$). The exposure in this region is defined by

$$X = \frac{dq}{dm} = \frac{1}{\varrho}\frac{dq}{dV} \qquad [2.49]$$

Thus exposure is measured by the charge produced by ionization at electron equilibrium in air of unit mass. Its *unit* is $C\,kg^{-1}$.

The earlier unit was the Roentgen (denoted by R). 1 R is the exposure that in 0.00129 g air (1 cm^3 of normal state) produces positive or negative ions carrying 1 electrostatic unit (esu) of electricity ($=3.34\times10^{-10}$ C) of either sign. Under the same circumstances 2.6×10^{-7} C positive and negative charges are produced in 1 g air.

$$1\ R = 2.6\times10^{-4}\ C\,kg^{-1}$$

It might well be asked why the ionizing effect produced *in air* is used for dosimetry. There are both theoretical and practical reasons for this. It should again be mentioned that ionization produced in air can in practice be measured fairly exactly, with good reproducibility and relatively simply. In principle it is also important to know that ionization produced in air runs parallel to the biological effects and is *independent of the wavelength* of the radiation. If the ionizing effects produced by radiation of two different wavelengths are the same in air, the biological effects in a given tissue under given conditions will also be practically the same. This holds only approximately (or not at all) for other effects, such as produced in a photographic emulsion or luminescence, since their wavelength-dependence is different from the wavelength-dependence of the biological effects. The degrees of darkening of photographic film, for instance, may be the same for radiation of different hardnesses, whereas the expected biological effects will be quite different. The correspondence between the effects of ionization in air and the biological effects is attributed to two circumstances. One has already been discussed (section 2.10.3; Fig. 2.27): the ratio of the absorption (and scattering) coefficients in the air and in the tissues is practically the same at various wavelengths. The other circumstance is the fact that the energy required to produce one ion pair is also independent of the wavelength; its value for electrons is approximately 34 eV in air (and on average is the same in the tissues).[5]

2.16.2. Biological dose. Dose equivalent

The biological effects of radiation depend not only on the absorbed energy, but also on the type of radiation and the nature (organ, tissue) of the irradiated target, and on such conditions as distance and duration. Consequently, in order to characterize the effect, a quantity covering all these factors must be used. This quantity is the biological dose. The determination of the biological dose poses a particularly complex problem in radiation therapy. The requirements are less strict in radiation

[5] Approximately 35 eV for protons, α-particles, etc.

protection, where the mean dose is accepted as sufficient. A quantity frequently used in radiation protection is the dose equivalent (H), which is defined as the product of the absorbed dose (D) and factors characteristic of biological effectiveness:

$$H = DQN \qquad [2.50]$$

where the quality factor Q is typical of the nature of the radiation, and all other factors are collected in N. Q may be regarded as a ratio expressing how many times more effective the radiation in question is from a biological aspect than X-radiation. Let D' denote the absorbed X-ray dose traditionally used in medical practice to attain a certain effect, and let D be the absorbed dose of the radiation in question required to produce the same effect. Hence

$$Q = \frac{D'}{D} \qquad [2.51]$$

For traditional X-radiation the value of Q is obviously 1 (by definition). It has approximately the same value for other ranges of X-radiation, and also for γ-radiation and β- (electron) radiation. For other types of radiation, however, the factor is larger than 1. This is a result of the fact that smaller doses of these types of radiation are required to produce the same biological effect as that of X-rays. Average values of quality factors are listed in Table 2.3.

Table 2.3

Average quality factors for various radiations

Radiation type	Q
X-radiation, γ-radiation	1
Electrons (>0.03 MeV)	1
Electrons (<0.03 MeV)	≈ 1.7
Slow neutrons*	3–5
Fast neutrons and protons (<10 MeV)	≈ 2
α-radiation, heavy nuclei, fission products	≈ 20

* Low-energy neutrons (<10 keV), mainly thermal neutrons

According to the international standards the value of the factor N is taken as 1, since an exact value has not been determined yet. Thus, the dose equivalent is equal to the product of the absorbed dose and the quality factor.

The *dose equivalent unit* is the sievert (denoted by Sv); its value is 1 if the biological effect is the same as that produced by an absorbed X-ray dose of 1 Gy.

The earlier unit, the *rem* (roentgen equivalent man), was defined as the absorbed dose of ionizing radiation which has the same biological effect as 1 rad X-radiation

$$1 \text{ rem} = 0.01 \text{ Sv} \qquad [2.52]$$

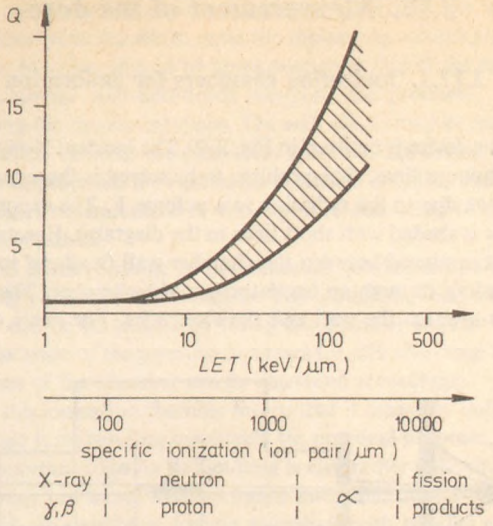

Fig. 2.48. Relation of linear energy transfer (LET) and quality factor (Q) for water

As concerns the quality factors of the various types of radiation, an interesting correlation can be observed in Fig. 2.48. The value of Q increases with the extent of linear energy transfer, which indicates that the linear ion density is of basic importance from the aspect of the biological effectiveness of radiation.

The dose equivalent is mainly used if the organism is exposed to radiation of various effectivenesses and it is desired to estimate the overall biological effect. The expected effect can be measured as the sum of the dose equivalents.

A given dose of some radiation may be taken up by the body during different times. From a biological aspect a knowledge of the dose rate is important; this quantity is defined as the quotient of the dose absorbed by the body and the duration of irradiation. Depending upon the dose type the units may be Gy h^{-1}, mGy h^{-1}, C kg^{-1} h^{-1}, etc. For instance, the absorbed dose rate at some region of the body is 1 unit if the absorbed dose per unit time is 1 Gy. Another derived concept is the *integral* or *volume* dose. If the dose is the same at every site in some part of the body of mass m (homogeneous dose distribution), the integral or volume dose is given by the product of the mass and the dose. Thus, the integral absorbed dose gives the energy absorbed by a tissue of mass m, for instance. The unit of the integral dose depends upon the dose actually used: J, C. Consider an inhomogeneous distribution in a sufficiently small domain within which the dose is constant; the integral dose is then calculated separately for every small domain, and the results are summed.

2.17. Measurement of the dose

2.17.1. Ionization chambers for calibration

This type of measuring device is outlined in Fig. 2.49. The incident X-rays or γ-rays are represented by the divergent continuous lines. The problem to be solved is the measurement of the charge q produced by the ionization due to the radiation in a volume V. The exposure is obtained from the quotient q/V (the volume is shaded with short lines in the diagram). For measurement of the charge q, an electric field is first produced between the chamber wall (made of some conducting material) and the electrode protruding through an insulator into the chamber. The electric field diverts the liberated charge carriers towards the wall and the electrodes. For every charge to be caught, the

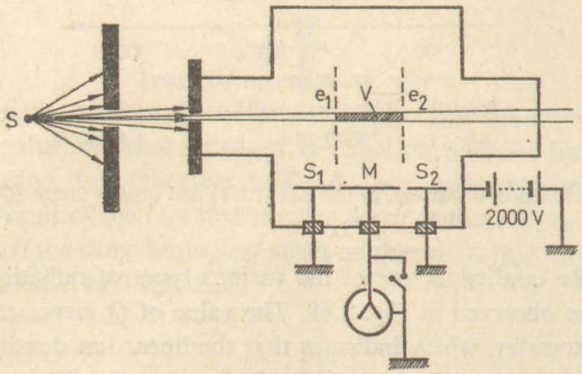

Fig. 2.49. Ionization chamber for absolute measurement of the exposure

recombination of charges of opposite sign must be avoided. This is done by applying a sufficiently high voltage (saturation current). The measurement starts with disconnection of the earthing of the electrode M (measuring electrode), whereby the electrometer becomes charged and measures the charge reaching the measuring electrode in a given period. The auxiliary electrodes S_1 and S_2 are used to produce a homogeneous electric field in the centre of the chamber from where the charges travel to the measuring electrode.

The measurement is correct if the number of charges is equal to that produced by the secondary electrons liberated in the volume V by the X-radiation or γ-radiation. This is emphasized for two reasons. First, some of the secondary electrons escape from the chamber and ionization is produced outside the chamber, with the result that some of the charges generated by ionization are not measured at all. Another point is that secondary electrons produced outside the chamber may enter it. In connection with the first possibility, it has to be taken into consideration that the collecting field of the measuring electrode also contains charges produced outside the volume V but still within the space denoted by the lines e_1 and e_2. These too are measured by the instrument. Consequently, problems are presented only by those charges produced by the electrons scattered outside the volume V to the left of e_1 and to the right of e_2. Though these are not measured, the loss is made good, since some of the secondary electrons produced in the left or right neighbouring regions enter the volume V and exert their ionizing effect in part there. The electrons passing through are thus compensated by the electrons entering the measuring volume (electron equilibrium is established).

Naturally, the chamber must be large enough so that the electrons do not strike the chamber walls (electrodes), since this could lead to two types of error: either the ionizing power of the electrons

is not fully utilized as a result of the collision, which means a charge loss, or the electrons impinging on the wall produce further electrons which enter the measuring volume and create a surplus charge. Another reason why the chamber should be large enough is that if the scattered X-radiation or γ-radiation reaches the chamber wall additional electrons are produced, which similarly results in surplus charge on entering the measuring space. The wall effects may be reduced, or even eliminated, if the chamber wall is made of some air-equivalent material. However, with calibrating chambers this cannot be done, for appropriate air-equivalent materials could be selected only if some method were known allowing exact measurement of the exposure according to the definition. This would be possible only after calibration.

The track length and effective range of the electrons produced by the radiation increase with the hardness (penetrating power) of the radiation. The harder the radiation, the larger the chamber required. This difficulty can be eliminated by filling the chamber with air to a pressure higher than 1 atmosphere. Such an increase of the pressure decreases the effective range of the produced electrons, and hence the dimensions of the chamber can be decreased accordingly.

The construction of this ionization chamber means that it measures only the exposure of primary radiation, and in principle it is therefore unsuitable for practical purposes, since the scattered radiation always plays an important role. As its handling is also rather difficult, the described type is used only in institutes carrying out exact measurements and calibrations. The chambers are made in various forms: standard air chambers, free-air chambers with parallel electrodes, etc. In medical practice small dosimeters are used; these are easier to operate, but they must first be calibrated with the chamber described above.

2.17.2. Small ionization chambers

Dosimetry in medical practice is mainly restricted to the measurement of photon and electron radiation. In this section, only the measurement of photon radiation is dealt with, though the results are also applicable to electron radiation. As concerns interaction with the medium, there is no basic difference between photon and electron radiation, since the effects actually measured with photons are produced by electrons.

It clearly follows from the foregoing that the dosimetry of photon radiation is particularly conveniently carried out by methods based on ionization of air. The use of the smallest possible ionizing chambers is advisable, since they virtually do not perturb the radiation field, and permit a relatively exact mapping of the spatial distribution of the radiation. A frequently used group of small ionization chambers are the thimble chambers (Fig. 2.50). One electrode is the chamber wall itself, while the other is a rod protruding into the chamber through an insulator. The measuring space is the air space of the chamber. Ionizing chambers can be used to measure either dose or dose rate. Their volume can be reduced below 1 cm^3. With this small size the device can be placed into the cavities of the body, thereby allowing direct measurement of the dose at certain sites within the body in treatment with X-radiation or γ-radiation.[6]

[6] For a more careful measurement of the dose distribution, phantoms are made of some appropriate material (water, wax, etc.); in shape and radiation absorption, these are similar to the part of the body to be irradiated. If the chamber is placed at various sites of the phantom, the dose distribution can be mapped.

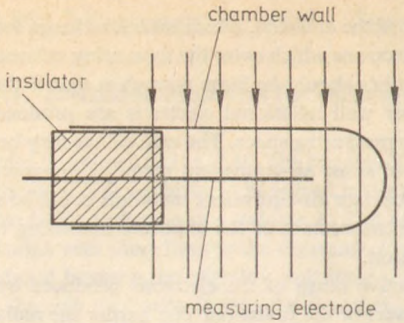

Fig. 2.50. Outline of the thimble chamber
The arrowed lines denote the radiation to be measured

By appropriate selection of the material and the chamber-wall thickness, the secondary electron density characteristic of either the air or the surrounding tissues is produced in the air space of the chamber. The former method is applied if photon energies up to 0.6 MeV are used, and the latter with photons of higher energy. For the lower energy range the chamber wall is made of air-equivalent material with a thickness large enough to prevent the secondary electrons produced in the surrounding tissues from entering the measuring volume. This condition can be satisfied with some suitable material with a wall thickness of 2 mm or less. (The chamber wall behaves as a compressed air layer within the chamber.) The higher the photon energy, the thicker the wall required for electron equilibrium characteristic of the air in the chamber to be established. Thick-walled and large chambers, however, are unsuitable. Accordingly, a different measuring principle is employed if photon energies larger than 0.6 MeV are to be measured. In this case the chamber wall is made so thin that secondary electrons produced in the surrounding tissues pass through it almost without attenuation. Consequently, a secondary electron density characteristic of the surrounding medium is produced in the small air space of the chamber.

The chambers developed for the lower energy range are *photon detectors,* whereas the high-energy chambers are *electron detectors.* The nomenclature is an indication that in the former case the measurement is based on the absorption of *photons* in the chamber (mainly the chamber wall), whereas in the second case the photons absorbed by the chamber are negligible, and it is rather the *secondary electrons* entering the chamber (from its surroundings) that are important.

The results with these two methods are discussed separately below.

1. The photon detector measures the exposure directly at a given site, from which the absorbed dose in air and in the surrounding tissues can be determined in a simple manner.

(a) *Absorbed dose in air.* Since the charge of an electron is 1.6×10^{-19} C, a 1 C kg^{-1} exposure is produced by the liberation of $10^{19}/1.6$ electrons, or by the same number

of ion-pairs. The production of a single ion-pair requires on average 34 eV, or $34 \times 1.6 \times 10^{-19}$ J; consequently, 34 J is needed to produce $10^{19}/1.6$ ion-pairs. Thus, a 1 C kg^{-1} exposure in air is equivalent to a 34 J kg^{-1}, i.e. 34 Gy absorbed dose. From this, however, it follows that for an exposure X the absorbed dose D_{air} is calculated from the equation

$$D_{air} = f_0 X, \quad \text{where } f_0 = 34 \frac{\text{Gy}}{\text{C kg}^{-1}} \left(= \frac{\text{J}}{\text{C}} \right) \qquad [2.53]$$

Since from the exposure the dose absorbed in air can be easily calculated, recently instead of exposure rather the dose absorbed in air is used and accordingly the instruments too are calibrated in Gy units.

(b) *Absorbed dose in tissues.* Since the absorptive power of tissues is greater than that of air, the photon radiation losing 34 J kg^{-1} energy in air, loses more in tissues. The absorbed energies in the case of photon detector are in the same proportion to each other as the respective *mass attenuation coefficients*, i.e.

$$\frac{D_{tissue}}{D_{air}} = \frac{\mu_{m,tissue}}{\mu_{m,air}} \quad \text{or} \quad D_{tissue} = \frac{\mu_{m,tissue}}{\mu_{m,air}} D_{air} \qquad [2.54a]$$

from which, with regard to [2.53]:

$$D_{tissue} = \frac{\mu_{m,tissue}}{\mu_{m,air}} f_0 X \qquad [2.54b]$$

2. With an **electron detector** one obtains directly the energy absorbed by the tissues by the Bragg–Gray principle measuring the charges liberated in the air space of the chamber, i.e. the energy absorbed by the air. Though the secondary electron density is the same in the chamber and in its surroundings, the absorbed doses are still different, because the stopping powers of air and tissues for electrons are different. The ratio of the absorbed doses is equal to the ratio of the *mass stopping powers* (S_m):

$$\frac{D_{tissue}}{D_{air}} = \frac{S_{m,tissue}}{S_{m,air}}, \quad \text{or} \quad D_{tissue} = \frac{S_{m,tissue}}{S_{m,air}} D_{air} \qquad [2.55]$$

Table 2.4 gives some mass attenuation coefficient ratios. If 1.08 is accepted as an average value, then according to [2.54b] at an irradiation dose of 1 C kg^{-1} the soft tissues absorb approximately 37 J kg^{-1}.

The Table also shows the radiation hardness-dependence of the energy absorbed by the tissues, at a given exposure, i.e. the wavelength-dependence of the proportionality of the exposure and the absorbed dose. Disregarding the extremely soft and the ultra-hard radiation (not presented in the Table) for *soft tissues* the proportionality is practically independent of the wavelength. This is to be expected from what was said above, as otherwise the two dose types could not be used together to characterize the biological effects. However, parallelism can exist between the biological

Table 2.4

Some data on the mass attenuation coefficient of tissues as related to air for photon radiation

Photon energy (MeV)	$\mu_{m,tissue}/\mu_{m,air}$	
	soft tissues	bones
0.1	1.07	3.54
0.2	1.08	2.4
0.4	1.1	1.25

effect and the absorbed dose, and between the biological effect and the exposure only if it also exists between the absorbed dose and the exposure. A substantial wavelength-dependence is observed only with the bones.

Table 2.5 contains data referring to the relative mass stopping power of carbon, but these yield information on the soft tissues as well.

Table 2.5

Some data on the mass stopping power of carbon as related to air for electrons

Electron energy (MeV)	$S_{m,carbon}/S_{m,air}$
0.1	1.016
0.3	1.007
1.0	0.985
3.0	0.946

2.17.3. Other methods of dose determination

Besides the methods based on the ionization of air, other radiation effects are also used in practice for dosimetry, e.g. photographic, luminescent, and more rarely thermal or chemical effects. In any actual case, however, the method most suitable for measurement of the radiation should be selected, with particular regard to the spectral distribution (in either the wavelength or the energy spectrum), the dose range, and so on. In the following section some remarks are made concerning the procedures used most frequently.

1. Film-dosimeters (Film-badges). The absorption spectrum of a photoemulsion differs from that of the tissues, film-badges displaying a considerable energy-dependence, which is especially strong with photon energies between 0.04 and 0.4 MeV. Consequently, photoemulsions are mainly used with *radiation sources exhibiting*

identical spectra. For instance, film-badges may readily be used to compare different radiation sources containing radium, but to compare the radiation emitted by for instance a radium preparation and an X-ray tube they can be employed only in conjunction with special evaluation methods. Persons who work with radiation are usually provided with film-badges, which are evaluated centrally.

2. Luminescence dosimeters. In these dosimeters the detector is a small plate made of some luminescent material enclosed in a light-proof case. The light produced by the radiation is transmitted through an optical system and mesaured with a photocell or a photomultiplier. The photocurrent yields information about the dose rate.

The disadvantages mentioned in connection with film-badges also hold for this method, except for those increasingly frequent cases when the dosimeters are made of air- or tissue-equivalent materials.

3. Calorimetric method. The essence of this method is the transformation of the absorbed radiation energy into heat, the absorbed dose rate being inferred from the temperature change. This method is mainly suitable for the measurement of X-radiation and γ-radiation. Its advantage is that it is independent of the *spectral distribution* of the radiation. A disadvantage, however, is the long time required to obtain the result, and the caution which must be practised, which makes the use of this method rather difficult in practice. The temperature changes due to the radiation are extremely small, and a smaller dose rate than 0.5 Gy/min is not measurable with this method. The temperature increase is of the order of magnitude of 10^{-4} °C.

4. Other methods. (a) *The electric conductivity* of some *semiconductors* increases on irradiation. The measuring instrument in the circuit is directly calibrated in dose units. Its small dimensions mean that the semiconductor crystal can be fixed on the end of a thin, flexible probe. This type of detectors can readily be introduced into the interior of the organism or into the cavities of the human body.

(b) Certain *solutions* and *gases* are also used, since the radiation induces *chemical changes* in them. Fe^{2+} oxidation (ferrous sulphate dosimeter) is frequently made use of, but the precipitation of iodine from alkyl iodides, the decomposition of formic acid, the bleaching of methylene blue, and other reactions are also applicable.

(c) *Some crystals* (e.g. alkali halides), *glasses* (e.g. phosphate glasses containing silver, glasses containing cobalt or lead), *organic solids* (e.g. plexi, PVC) and so on become coloured on irradiation. (This is the effect which can be observed on the glass coating of X-ray tubes after prolonged use.) The intensity of the coloration is measured by means of colorimetry. The coloration effects on consecutive irradiations are additive. The colorimetric measuring light has practically no effect on the irradiation-induced colour, and hence the evaluation can be repeated several times. Since the coloration can be eliminated by heating, the same crystal can be repeatedly used.

(d) Appropriately activated glasses can be used in a different way too. It is well known that certain substances which have previously been exposed to X-radiation or γ-radiation become luminescent in the visible range if excited with ultraviolet light. The emitted luminescent light, measured for instance with a photomultiplier, is proportional to the X-ray or γ-ray dose *(photoluminescence dosimeter).*

(e) Certain substances (e.g. anthracene) which are luminescent if irradiated with ultraviolet light gradually lose their luminescence if exposed to X-radiation or γ-radiation. *Luminescence degradation dosimeters* are based on this phenomenon.

(f) A number of materials (e.g. manganese-doped CaF_2, LiF crystals) emit light on heating, after previous X-irradiation or γ-irradiation. This effect is utilized in *thermoluminescence dosimeters.*

The above effects are used in practice in different dose ranges (Fig. 2.51). Individual pocket dosimeters are made as small cubes, needles or foils, and are carried suitably encased either in the pocket or round the neck.

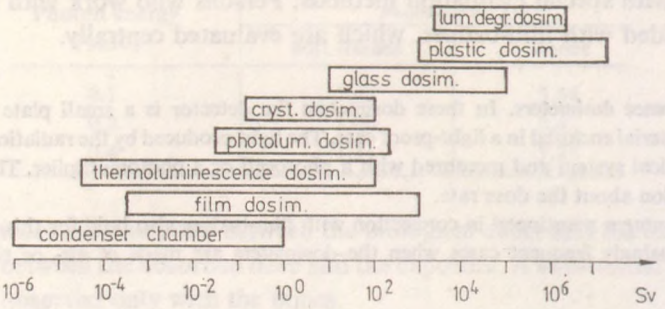

Fig. 2.51. Application ranges of various dosimeter types. Capacitor chambers are ionization chambers used in individual dose measurement

2.18. Radioactive isotopes as tracers[7]

Radioactive isotopes disclose their presence by their radiation, and thus their movement and fate can be traced *(tracers)*. The radioactive isotopes form compounds in the same way as the stable isotopes. This permits the production of labelled molecules and compounds, which behave virtually identically to the unlabelled ones in the various chemical, biochemical and biological processes. Besides the simpler inorganic labelled compounds, a very wide range of labelled organic compounds exist (labelled amino acids, hormones, pharmaceutical products, etc.). Moreover, it is also possible to exchange the $^{12}_{6}C$ atom by the $^{14}_{6}C$ isotope at *a fixed site in a molecule* containing several carbon atoms. In many instances the simple labelled compound is incorporated into a living organism, where it is transformed into a more complex organic molecule by the normal function of the organism *(biosynthesis)*.

2.18.1. The importance of radioactive isotopes as tracers

(a) Radioactive isotopes introduced into an organism (a chemical or other system) are distinguishable by their radiation from the atoms already present. This permits the relatively simple acquisition of *information* about the *dynamics of processes* of uptake, incorporation, exchange, secretion, etc. Many problems can only be solved by this means.

[7] The radiophysical data on the isotopes dealt with in this chapter are given in Table 8.14, Chapter 8.

(b) The tracer method is extremely *sensitive*. The lowest limit of the most sensitive microanalytical technique is of the order of 10^{-11} g, which requires the simultaneous presence of 10^{10}–10^{12} atoms. With up-to-date radioactive methods, on the other hand, in principle even the presence of only one atom can be detected; at any event, 10^5–10^6 atoms are sufficient for quantitative measurement. It follows that the sensitivity of the radioactive method is 6–8 orders of magnitude higher than that of microanalytical methods.

(c) The high sensitivity allows the study of various processes with amounts of substances so small that they have practically no influence on the life processes. For instance, the mass of 0.4 MBq radioactive $^{131}_{53}I$ necessary for examination of the thyroid function is approximately 8×10^{-11} g. This small quantity disturbs neither the normal functions of the organism, nor the state of the thyroid gland. The presence of the radioactive decay products is in most cases negligible. For instance, the $^{131}_{54}Xe$ produced by the radioactive decay of $^{131}_{53}I$ has no disturbing effect at all. With appropriate doses, the biological effects of the radiation of the isotope are similarly of no consequence.

Not only radioactive but also *stable isotopes* may be used for tracing. In principle the method consists in changing the ratio of the isotopes as compared to the ratio existing in nature. As a result, certain properties of the labelled substances (e.g. density, viscosity, melting point, infrared absorption spectrum, etc.) are changed and hence become detectable. A mass spectrometer may also be used, since mass spectrometry allows direct determination of the isotope ratio. However, these methods are less convenient and less exact than the methods based on radiation, and are applied only if suitable radioactive isotopes are not available. As examples, the elements nitrogen and oxygen, which are important in biological experiments, have no radioactive isotopes with a half-life longer than 10 minutes. Consequently, radioactive tracing experiments can be carried out with these isotopes only under exceptional circumstances. Nevertheless, if their labelling is important, stable isotopes may be employed instead of the radioactive ones. The stable isotopes most frequently used are $^{2}_{1}H$, $^{15}_{7}N$ and $^{18}_{8}O$.

2.18.2. The possibility of tracing with isotopes

The examples given below are naturally from the field of medical application.

(a) *Application at a molecular level.* The mechanisms of many of the complex *chemical reactions* (synthesis, decomposition of various substances) occurring in living systems may be elucidated with the use of radioactive isotopes. In investigations carried out to reveal the genetic code, for instance, the meaning of the UUU triplet was solved by adding poly-U as synthetic mRNA to as many cell-free protein-synthetizing systems as the number of the existing amino acids. Each of these cell-free systems contained not only the mRNA, but also all the amino acids required for protein synthesis, but in every system a different amino acid was labelled with $^{14}_{6}C$. Based on the information carried by the poly-U the same polypeptide was synthetized in all the systems, but the product was radioactive only in the system in which phe-

nylalanine was labelled. It could be proved by this series of experiments that the UUU triplet corresponds to the genetic code of phenylalanine. In even more complex experimental systems the codes of the other amino acids were elucidated by means of similarly applied radioactive amino acids.

Labelling with radioactive isotopes is frequently used in *pharmacokinetics*. The uptake, distribution and elimination processes of drugs are easily followed via the tracer method. From a knowledge of the site and rate of decomposition and binding, information is obtained about the mechanism of action.

Processes occurring essentially at a molecular level can be followed by the *radioimmunoassay* method, which combines the high specificity of immune reaction and the sensitivity of the isotope technique. The method is applicable for the independent determination of the concentrations of small quantities of various substances with similar chemical structures in suitable solutions (e.g. hormones in the serum). The method consists in adding the serum containing the hormone to be studied (the antigen) to a solution which contains the specific antibody and also a known quantity of the antigen labelled with radioisotope. The labelled and unlabelled antigens compete with each other with the same probability for the binding site on the antibody. After a sufficient incubation period the labelled and unlabelled antigens in the product antigen–antibody complex are bound to the antibody in the same ratio as they were initially present in the solution. Consequently, the quantity of the antigen can be determined from the specific activity of the complex. This method permits the determination of 10^{-9}–10^{-12} g amount of substance, and has the additional advantage that the investigation can be carried out *in vitro;* thus, no isotope is administered to the patient.

(b) *The study of time-dependent processes.* Among the first diagnostic applications of radioisotopes was the use of radioiodine in the diagnostics of the thyroid gland. For this purpose the γ-radiation of $^{131}_{53}\mathrm{I}$ is measured. One of the most frequent objectives is the determination of the *iodine-uptake capacity of the thyroid gland.* From the time-dependence of the iodine uptake of the thyroid gland, useful information is obtained about the function of this organ. For examination, 0.2–0.4 MBq iodine isotope is administered orally into the organism, in the form of NaI solution. Subsequently, the quantity of iodine taken up in consecutive time periods is measured with a scintillation counter placed close to the thyroid gland. Figure 2.52 depicts *the iodine accumulation curves* of a healthy and a pathological thyroid gland. Valuable information may also be obtained by determining the *radioactive iodine quantity* secreted by the thyroid gland into the circulation. A blood sample taken from the patient 2 and 48 hours after the administration of radioactive iodine is similarly measured with a scintillation counter. This measurement is best carried out by placing the test-tube containing the sample into a cavity in a scintillator crystal (a *well-type crystal*). The sample is surrounded by the detector; this increases the efficiency of the measurement considerably. Valuable information is obtained about the function of the thyroid gland by examining *the composition of the serum.* The different hormone

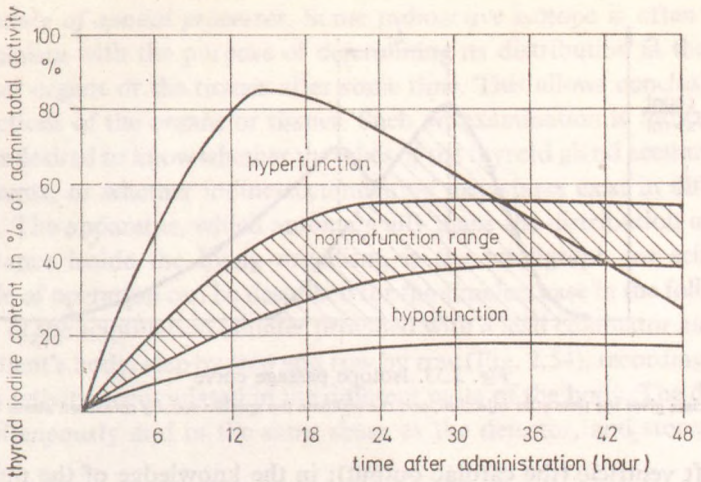

Fig. 2.52. $^{131}_{53}$I uptake and accumulation of the thyroid gland under healthy and pathological conditions

fractions (diiodo-tyrosine, monoiodo-tyrosine, thyroxine, triiodo-thyronine) are separated by chromatography, and the isotope concentration of each fraction is determined by some radiation detection method (radiochromatography). More recently, via the radioimmunoassay method the quantity of thyroid hormones in the serum can be determined without their previous separation.

Renography is another example for the examination of the function of an organ. In order to study the renal function, a scintillation detector is placed above each kidney. The radioactive substance labelled with a γ-radiating isotope (e.g. hippuran labelled with $^{125}_{53}$I) is administered intravenously, and the rates of accumulation and secretion of the substance by the kidneys are recorded continuously. The resulting *renogram* yields information on the state of the kidneys. Whereas measurement of the iodine accumulation by the thyroid gland requires a few days, examination of the renal function with a radioisotope takes approximately half an hour.

Radiocirculography deals with relatively fast processes. An isotope (e.g. human serum albumin labelled with 2–5 MBq $^{131}_{53}$I) introduced into the circulation (e.g. by intravenous injection) yields data characteristic of the circulation and of the cardiac function. If the detector placed above the heart is provided with a collimator which detects simultaneously the isotope passing through both ventricles, a *double peaked* curve is obtained (Fig. 2.53). The first peak refers to the passage of the isotope through the right ventricle, and the second to that through the left one. The time interval between the two maxima is the *mean pulmonary circulation time* (t_c). The measurement of the curve presented in the diagram generally lasts 20–50 s. If the recording is continued, another, more protracted curve is obtained, which indicates the repeated arrival of the isotope into the heart (recirculation).

Without going into details, it will be mentioned only briefly that the outlined method permits determination of the blood volume flowing per minute into the aorta

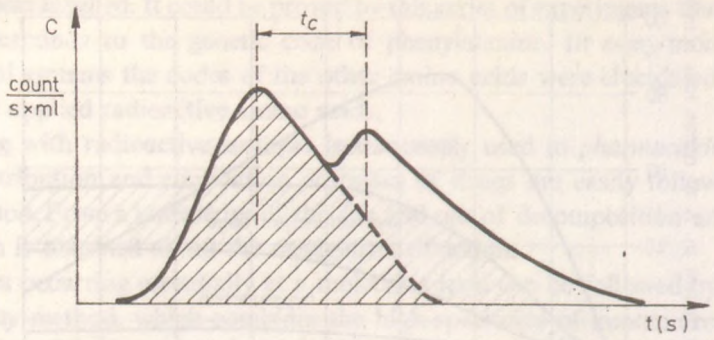

Fig. 2.53. Isotope passage curve

The abscissa gives the time after injection, and the ordinate the specific activity measured above the heart

from the left ventricle (the cardiac output); in the knowledge of the pulse rate, the *volume per pulse* and *the volumes of the ventricles* too can be obtained. In principle, other circulation characteristics (pulmonary circulation, limb circulation) may be determined in a similar way. Recently, the majority of these types of examinations are carried out with γ-cameras (see below).

The decomposition of red blood cells and their regeneration may be followed by means of their labelling. (Similar examinations may also be carried out with other cells or tissues.) The activity of labelled red blood cells introduced into the blood decreases with time. This decrease is in part due to the physical decay of the labelling isotope (e.g. $^{51}_{24}Cr$), and in part a result of the spontaneous decomposition or destruction of the red blood cells. If blood sampling is carried out over a prolonged period (e.g. 30–40 days), and the number of counts for each sample is plotted as a function of time, the time at which a certain proportion (e.g. 50%) of the red blood cells have been destroyed can be read off the curve obtained. The biological half-life of red blood cells circulating under healthy conditions is found experimentally to be 30 days. Thus, from the decrease in activity of the labelled red blood cells, conclusions may be drawn concerning their decomposition. On the other hand, if radioactive iron (e.g. $^{59}_{26}Fe$) is injected intravenously into the organism, continuous blood sampling reveals a progressively increasing $^{59}_{26}Fe$ activity of the red blood cells (since the iron getting into the bone marrow is continuously incorporated into the newly forming red blood cells). In this way, the $^{59}_{26}Fe$ contents of the blood samples provide information on the regeneration and formation of the red blood cells. Frequently, both isotopes ($^{51}_{24}Cr$ and $^{59}_{26}Fe$) are applied simultaneously to study this haematological problem. The blood samples then contain both isotopes *(double labelling)*, which must be determined independently. The measurements are carried out with a scintillation counter provided with a differential discriminator (sections 5.6.2 and 2.15): the number of pulses produced by the sample in a hollow crystal is first determined with parameters corresponding to the γ-energy of $^{51}_{24}Cr$, and subsequently with the parameters of the γ-energy of $^{59}_{26}Fe$.

(c) *The study of spatial processes.* Some radioactive isotope is often introduced into the organism with the purpose of determining its distribution in the organism, the individual organs or the tissues after some time. This allows conclusions on the state or functions of the organs or tissues. Such an examination is indicated for instance, if it is desired to know whether the lobes of the thyroid gland accumulate iodine to equal extents, or whether iodine-accumulating metastases exist in different parts of the body. The apparatus, which automatically maps the distribution of the radioactive substance inside the living organism, is the scintigraph (or scintiscanner). The principle of operation can be described for the simplest case in the following way: the detector of the scintillation counter provided with a lead collimator automatically scans the patient's body, step by step and row by row (Fig. 2.54), recording from point to point the activity accumulated in the different parts of the body. The drawing unit moves simultaneously and in the same sense as the detector, and stores the pulses

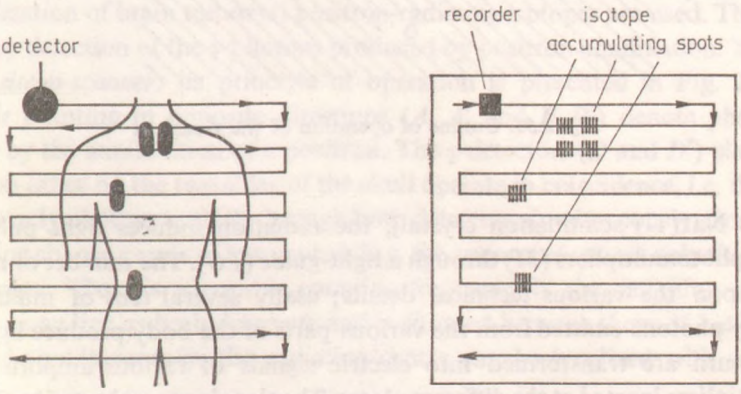

Fig. 2.54. The principle of the scintigraph

either electromagnetically or photographically. By this means a map is made of the human organism, which demonstrates where the isotope accumulates to a higher or lesser extent.

In the examination of the isotope distribution, imaging methods by means of *fixed detectors* have recently come into the limelight. The detector surface applied in this method is large enough to make any motion of the detector unnecessary. The detector, placed in a fixed position over the part of the body of interest (e.g. the liver, heart, brain, lungs), detects the activity distribution and its variation in time. This method is employed for instance, with the *gamma-camera* (see Fig. 2.55, Supplement).

Figure 2.56 outlines the principle of operation of the apparatus. The γ-radiation emitted from the various sites of the organ (O) to be examined impinges on a multichannel lead collimator (Pb), which allows past only those rays nearly parallel with the channels. In the detector (D), approximately 30 cm in diameter and 1.0 cm thick

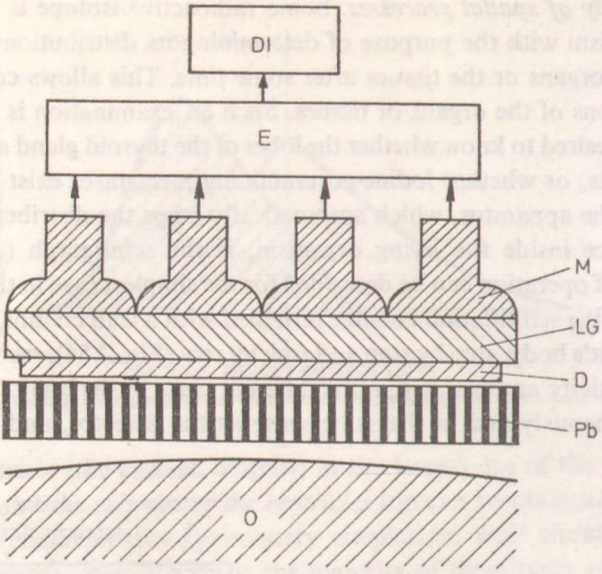

Fig. 2.56. Outline of operation of the γ-camera

[usually a NaI(Tl) scintillation crystal], the radiation induces light pulses which reach the photomultipliers (*M*) through a light-guide (*LG*). The number of multipliers depends upon the various technical details; usally several tens of multipliers are used. The γ-photons emitted from the various parts of the body produce light pulses, which in turn are transformed into electric signals of various amplitudes in the photomultipliers located at the different places. The signals are evaluated by the electronic unit (*E*) connected to a computer, and from the amplitude distribution the unit establishes the site of γ-photon emission; subsequently, using the data obtained, the computer stores the activity distributions at the consecutive points of time. The stored information can be used in two ways: (1) the isotope distributions at different times are displayed on a black and white, or a coloured screen (static display); (2) the time-dependence of the isotope content of the organ, or some specified part of it (region of interest: ROI) is displayed in the form of a diagram *(dynamic examination)*. Particularly this latter method represents a qualitatively new technique in contrast to the older ones: when sufficient isotope quantities are applied, even the distribution changes at 0.1 s intervals can be detected. This allows the study of very fast processes (e.g. cardiac action, cerebral circulation). Thus, the γ-camera is equally able to follow space- and time-dependent processes. For labelling, isotopes emitting relatively soft or medium hard γ-radiation are used (in most cases $^{99m}_{43}Tc$). One advantage of soft radiation is that the walls separating the collimator channels can be made of thin lead, which in turn permits a large channel density. A further advantage is the possibility of applying thin detector crystals. These factors favourably influence the spatial resolution.

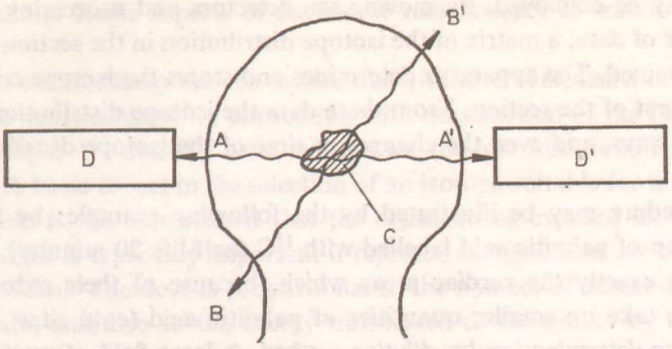

Fig. 2.57. Scheme relating to the positron scanner

For the detection of isotope accumulation within the organism, in some instances (e.g. localization of brain tumours) positron-radiating isotopes are used. This method is based on detection of the γ-photons produced by positron annihilation. This device is the *positron-scanner;* its principle of operation is presented in Fig. 2.57. Each arrow-pair pointing in opposite directions (A, A' and B, B') denote photon pairs produced by the annihilation of a positron. The γ-detectors (D and D') placed opposite to each other on the two sides of the skull operate in coincidence, i.e. the apparatus counts only photons passing through both detectors simultaneously. Consequently disregarding chance events of low probability, the apparatus counts only the annihilating positrons. There is a notable counting frequency if the radiating centre (C) lies in the same line as both detectors and is situated between them. If both detectors are moved simultaneously, the radiating centre can be localized with satisfactory accuracy.

In a recent development a γ-camera is used as one detector, while the other is a small-surface focus detector. Similarly as with the device described above, coincidence operation allows exact localization of the site of positron decay. This equipment simultaneously records the isotope distribution of a comparatively large part of the body, and as a result of its construction combines the advantages of the γ-camera and the positron scanner.

Mention may be made here of a procedure recently developed for examination of processes in space and time. The method is called *emission computed* tomography. As the name indicates, this yields information about the distribution of radioisotopes introduced into the organism by the detection of the radiation *emitted* by the isotope and the *methods of computed X-ray tomography* (cf. section 2.12). (Computed tomography involving an external X-ray source is called *transmission X-ray tomography* to distinguish it from the tomography described in this section.) In emission computed tomography an internal radiation source (some γ- or positron-emitting isotope) is used. Either one or two wide-angle γ-cameras are used for detection, or several small-surface scintillation or gas ionization detectors placed concentrically around

the body may be employed. By moving the detectors and processing a sufficiently large number of data, a matrix of the isotope distribution in the section under examination is obtained. The apparatus determines and stores the isotope content of each volume element of the section. From these data the isotope distribution is displayed in the usual ways, and even the changes in time of the isotope distribution may be recorded.

The procedure may be illustrated by the following example: by means of the administration of palmitic acid labelled with $^{11}_{6}C$ (half-life 20 minutes), it is possible to determine exactly the cardiac areas which, because of their reduced function, for instance, take up smaller quantities of palmitic acid (cold sites).

(d) *Volume determination by dilution method.* A large field of application is the use of radioisotopes in *volumetric* methods. A radioactive solution of known volume (V_0) and specific activity (c_0) is added to a liquid of unknown volume (V) and the mixture is homogenized. A sample is taken from the mixture and its specific activity c is determined. It is obvious that

$$V_0 c_0 = (V_0 + V)c, \quad \text{i.e.} \quad V = V_0 \left(\frac{c_0}{c} - 1\right) \qquad [2.56]$$

Since V_0 is in most cases negligible as compared with V, the unknown V is given by $V_0 c_0/c$.

Determinations of volume, and of the *distribution volume* in living organisms are based on a similar principle.

For determination of the *circulating blood volume,* labelled red blood cells are first prepared, e.g. $^{32}_{15}P$ or $^{51}_{24}Cr$ is added to 5–10 ml blood taken from the patient. Within 1–4 hours, a considerable proportion of the isotope is incorporated into the red blood cells, which thereby become labelled. The labelled blood is reinjected into the circulation. After 15–20 minutes (when the injected blood is homogeneously mixed with the circulating blood), a blood sample is taken, its specific activity is measured, and the total circulating blood volume is determined via [2.56]. If the haematocrit value is known, the red blood cell volume may be obtained in a simple way. However, it is also possible to label blood through the plasma (e.g. by administering human serum albumin labelled with $^{131}_{53}I$). The circulating blood volume and plasma volume are obtained similarly as above.

Determinations of the distribution volumes of the various substances in the organism are carried out in a similar way, e.g. the sodium volume, with $^{24}_{11}Na$, the chlorine volume with $^{38}_{17}Cl$, and so on.

The total water volume of the organism may be determined with tritiated or heavy water. Since tritium is a radioisotope, the measurement is carried out similarly as in the above example, but since deuterium is a stable isotope, densitometry is performed here.

2.18.3. Some aspects of the use of radioisotopes as tracers

(a) Certain examinations may be carried out with several elements or with several isotopes of the same element. In connection with examination of the thyroid gland, the iodine isotopes ^{131}I and ^{125}I were referred to, though other iodine isotopes too may be used. A basic aspect in the selection of an isotope suitable for the solution of a given problem is the requirement that the organism be exposed to the *minimum possible dose*. This is especially important if repeated examinations are to be made in the same individual. The dose is proportional to the number of atoms disintegrating in the organism, and also to the energy transferred to the tissues by the radiation produced in the disintegration of a single atom. It is therefore advantageous to use isotopes of *short half-life* (of the order of minutes or hours) which interact with the tissues mainly through γ-photons, since they are absorbed to a smaller extent.

The importance of the half-life is obvious if the decay law (see [2.31]) is written in the form

$$A \sim \frac{N}{T} \qquad [2.57]$$

The activity A can be measured with sufficient accuracy with a given apparatus only if it is above a certain limiting value. It follows from [2.57] that a smaller number (N) of radioactive atoms of short half-life (T) are required to attain a certain activity. Consequently, the introduction of a smaller quantity of a shorter half-life isotope into the organism is sufficient. One examination may be carried out with variously labelled compounds. With the selected isotope it is preferable to label a compound with a short biological half-life (e.g. a few hours).

(b) Another aspect in the selection of a suitable isotope concerns the manner *of decay* and *the type of radiation emitted*. In the case of tracer isotopes the radiation is of importance as regards detection. γ-radiation emerging from the organism with sufficient energy is generally used for detection. In the tracing technique, therefore, corpuscular radiation, even if stopped by the tissues, is unnecessary and indeed to be avoided. For this reason, if possible, only those isotopes are used whose dose load is practically due to their γ-radiation alone. (Isotopes which decay by shell electron capture are also suitable, since these are detectable by their X-radiation.)

In order to spare the organism from radiation, *harder* γ-radiation is more favourable. However, for technical reasons isotopes emitting relatively *soft* radiation have to be used, since in the measurement it is important that as much as possible of the radiation be absorbed by the detector [e.g. a NaI(Tl) crystal a few cm thick]. As a compromise, 0.1 MeV photons are frequently used.

A favourable isotope as concerns the above conditions is $^{99m}_{43}Tc$, which emits 140 keV γ-photons with a half-life of 6 hours. Though some of the photons undergo internal conversion, the energy of the conversion electrons is also predominantly transformed into photon energy (characteristic X-radiation). Technetium may be

used to label a large number of compounds. For the solution of a given problem, the most suitable compound is selected, one aspect being a short biological half-life.

Short half-life isotopes are applied for tracing only if they can be obtained at the place where their use is desired. This is possible with the isotopes dealt with in this section. The isotope $^{99m}_{43}Tc$ is produced from the mother element $^{99}_{42}Mo$ by negative β-decay, while $^{113m}_{49}In$ is obtained from $^{113}_{50}Sn$ by K capture. The equipment producing these isotopes is a *technetium or indium generator*.

Some health institutions equipped with *cyclotrons* produce very short half-life isotopes. This has opened up further possibilities of medical examinations (cf. section 2.14.7). Some of the isotopes produced by the cyclotron are $^{11}_{6}C$ (20.4 min), $^{15}_{8}O$ (2.1 min) and $^{13}_{7}N$ (10 min). These are elements of great importance in both medical research and medical practice since they are integral constituents of the living organism. Oxygen and nitrogen isotopes with longer half-lives are not known, and consequently these radioactive isotopes can be used only in the way described. The examples given are all positive β-decaying isotopes. Positron-radiating isotopes of short half-life are also used as tracing radioisotopes, for measurement of the annihilation photons flying in opposite directions permits localization of the radiation source with high accuracy.

2.19. Ionizing radiation and the living organism. Radiation hazards and chemical hazards

1. Basic phenomena. Ionizing radiation damages the living organism. This effect is primarily produced by the resulting *ionization* (and *excitation*) in the organism. The charged particles cause ionization directly, while X-ray and γ-photons and neutrons do so indirectly. The ionization may occur in the molecule playing the key role in the development of the hazard, or in the water molecules comprising the bulk of the mass of the living organism. The former case is usually referred to as a *direct*, and the latter as an *indirect* radiation effect. In the indirect case, monovalent free hydroxyl radicals may be formed from water molecules in contact with the air; these free radicals undergo intermediate processes and are finally transformed to hydrogen peroxide. Free radicals migrate by diffusion; the result of both direct and indirect radiation effect may therefore appear in one or another of the biologically important molecules in the form of some lesion which changes the biochemical reactions or produces mutations resulting in damage, and possibly in the killing of the cells or even the whole organism. The injury sometimes develops only after a latency period of even several years; this is *delayed radiation damage*. These processes are depicted in Fig. 2.58.

A basic role in radiation damage of cells can be attributed to the effect on DNA. The DNA molecule is several orders of magnitude larger than any other molecular component of a cell, so that molecular damage by ionization occurs with the greatest

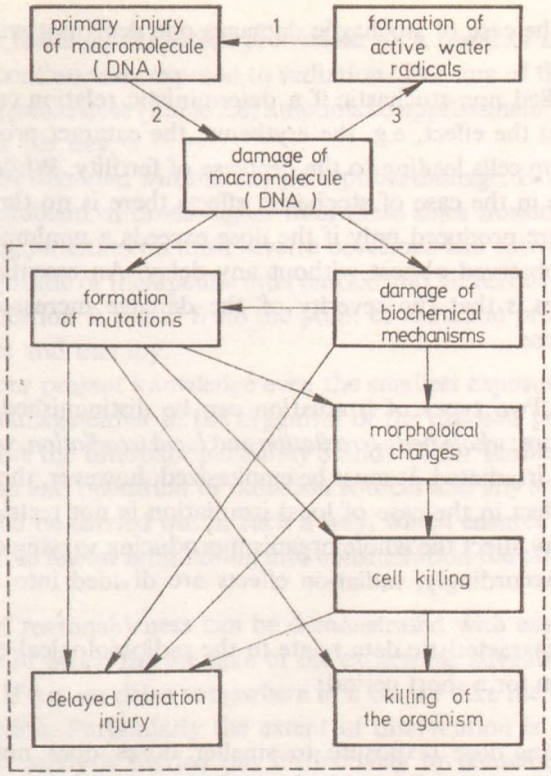

Fig. 2.58. Scheme of the processes connected with the development of biological radiation effects
Arrows 1 and 3 refer to the reactions between active water radicals and macromolecules, arrow 2 to the intramolecular energy transfer, and unmarked arrows to metabolic processes. The series of processes following the primary radiation effect can be seen in the frame

probability in DNA or its surroundings. The functional consequences of the molecular damage manifested at a cell level are of decisive importance in the case of DNA. As regards haploid cells, some chromosomal fragment in a single cell occurs in at most two copies, and the damage of either may result in the loss of some given cellular function. On the other hand, damage to any molecule which exists in several thousand copies in the same cell is less harmful.

Based on the ever increasing experience, but also merely from the reasoning outlined above and with respect to Fig. 2.58 it seems to be justified to divide radiation effects into two groups. One kind of classification discriminates genetic and somatic damages. The most recent categorization makes a distinction between *stochastic and non-stochastic effects*. The first group comprises effects occurring at a given dose with a certain probability in a random-like way; this probability increases with increasing dose. The size of the dose influences the probability of the occurrence of a damage, not its severity. Such stochastic effects are e.g. mutations and genetic consequences in general and the development of malignant diseases as well. It can be seen from the

examples that in the case of stochastic damages one deals not with acute, but with delayed effects.

The effect is called non-stochastic if a deterministic relation can be observed between the dose and the effect, e.g. the erythema, the cataract produced on the eye, the damage of germ cells leading to the decrease of fertility. While according to our present knowledge in the case of stochastic effects there is no threshold dose, non-stochastic effects are produced only if the dose exceeds a minimum threshold value and they can be observed almost without any delay. An essential feature of non-stochastic processes is that the severity of the damage increases with increasing above-threshold dose.

2. Dose levels. Two types of irradiation can be distinguished according to the extension of exposure: *whole body irradiation* and *local irradiation,* when only a smaller part of the body is irradiated. It must be emphasized, however, that the development of the radiation effect in the case of local irradiation is not restricted to the site of absorption, but may affect the whole organism producing varying degrees of damage to its functions. Accordingly, radiation effects are divided into *local* and *general* effects.

The following characteristic data relate to the radiobiological effects of exposure to γ- or X-radiation for a short period:

0.25 Sv: limiting dose (exposure to smaller doses does not generally cause any clinically observable damage);
0.5 Sv: small decrease in the number of lymphocytes;
0.75–1.0 Sv: *critical dose* (passing indisposition, fatigue);
1–2 Sv: stronger, more prolonged lymphopoenia, rarely death;
2 Sv: decrease of the cellular elements in the blood, death in ca. 15%;
4 Sv: half-lethal dose (50% mortality within 30 days);
6 Sv: lethal dose (100% mortality).

Approximate doses in exposure to X-radiation applied in medical practice:

Chest X-raying with luminescent screen	10– 50	mSv
Chest X-raying with X-ray film	ca. 10	mSv
Chest X-raying with fluorography	ca. 1	mSv
Dental X-ray photograph	15–100	mSv
Abdominal X-raying with luminescent screen	100–150	mSv
Treatment of malignant tumours (local irradiation)	30– 70	Sv

The use of an image intensifier may reduce the diagnostic doses by several orders of magnitude.

3. Background radiation. Radiation protection. As a result of natural background radiation man is continuously exposed to radiation. The sum of the different components of the background dose (Table 2.6) amounts to approximately 1.4 mSv annually, which is 3.5–4 μSv per day.

This dose can be tolerated without any perceptible damage. However, it is justified to deal with the problem of doses higher than these since nowadays people may be exposed to ionizing radiation on most diverse occasions and activities. Such occasion may be e.g. a diagnostic or therapeutic intervention and numerous occupational activities. Here the question is raised from the point of radiation protection rather than that of diagnostics and therapy.

According to our present knowledge even the smallest exposure to radiation may cause biological damage either in the organism of the exposed person or in the descendants. Therefore the International Safety Standards for Radiation Protection rule that the design, use and operation of radiation sources and any activity accompanied by radiation should be carried out in such a way, which ensures a level of exposure below the reasonable lowest limit taking into consideration the economical and social factors.

The concept of reasonableness can be demonstrated with examples. Human life is full of activities in which for the sake of the expectable advantages or benefits one has to take risks. If e.g. we drive somewhere in a car we take the risk of the travel to reach our destination. Particularly the extent of intervention is often decided by a risk versus benefit analysis carried out instinctively or consciously. This situation arises frequently in surgical activity but also in drug treatment and in the diagnostic and therapeutic applications of radiations. With regard to radiation protection international recommendations, too, apply the risk–advantage, risk–benefit point of view.

Table 2.6

Distribution of dose equivalents obtained yearly from background radiation

Origin of radiation	Critical organ	Quantity in the whole body (kBq)	Dose equivalent per year (mSv)
Cosmic radiation	whole body	–	≈ 0.4
Environmental radiation	whole body	–	0.5 – 1*
			1.0 – 4**
Incorporated ^{40}K	muscles, whole body	≈4	≈0.2
Incorporated ^{14}C	fatty tissue, whole body	≈4	≈0.02
Incorporated ^{226}Ra	bones, haematopoietic organs	0.2 – 0.02	0.1 – 0.5
Incorporated ^{222}Rn	lung	–	0.3 – 2.5

*in open air

**referring to radiation in houses and depending on building materials

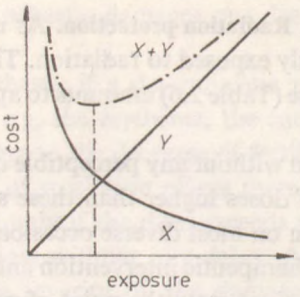

Fig. 2.59. Optimalization of radiation protection

The reasonableness or more exactly the *optimizing requirements* are shown in Fig. 2.59. For easier understanding let us think of a laboratory where the staff works with radiating substances. The total radiation exposure of the working team is indicated on the horizontal axis and the cost involving accidental health detriment of the workers and radiation protection is given on the vertical axis. The lower is the exposure permitted the higher will be the cost of protection, but the risk will be smaller, consequently the cost of health detriment will be also smaller. The first relationship can be considered nearly hyperbolical, the latter one linear and they are denoted in the figure by X and Y respectively. The total cost is the sum of the two partial costs. The minimum of the resulting $X+Y$ curve determines the reasonable total exposure.

The outlined optimizing analysis cannot be carried out in an exact way in every respect, thus one has to be satisfied with qualitative or semiquantitative approximations.

Beside the optimizing principle up-to-date radiation protection finds it necessary to give limits which cannot be exceeded. According to international rules valid at present *the annual dose equivalent limit at occupational exposure is 50 mSv*. The annual dose equivalent limit for the individual organs and tissues is 500 mSv. The human eye lens is an exception, with a limit of 150 mSv. Young people below the age of 18 years must not be exposed to occupational radiation hazards.

4. Radiation hazards and chemical hazards. In everday life we come into contact with numerous chemicals which may be biologically hazardous. The injury starts with molecular interactions, but the consequences may be manifested at cell, tissue or even organ level. The processes induced by radiation and by chemicals are in many instances similar, differing only in the initial steps of the process. Even here, however, similar phenomena are known, for instance the strand breaks induced by ionizing effects or chemical agents in the nucleic acids, which are of fundamental importance from the aspect of hazardous effects. However, the differences displayed at a molecular level gradually disappear at higher levels. Hence, any of the physical or chemical agents may produce mutations, malignant transformations, cell death, or cell aging.

A scheme of the processes induced by chemical substances is presented in Fig. 2.60. This demonstrates that only some of the hazardous chemicals are active, i.e. only a proportion of them develop direct interactions, the alkylating agents. Another group of chemicals become hazardous only if they are previously activated by metabolic processes, e.g. benzopyrene or ethylene. On the other hand, active chemicals may also be inactivated by metabolic processes.

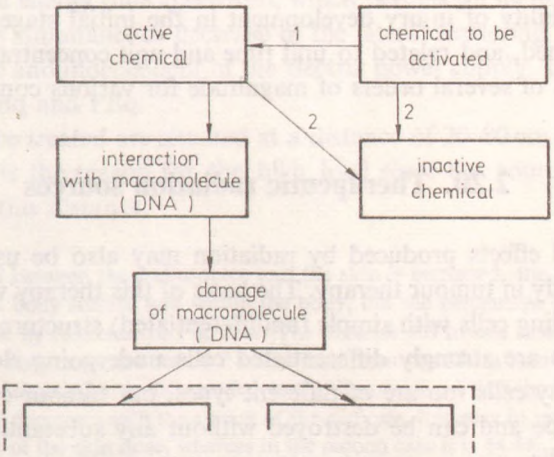

Fig. 2.60. Scheme of the development of chemical damage

Arrows 1 and 2 indicate the activation and inactivation possibilities during metabolic processes. Processes following the primary processes are only referred to (see Fig. 2.58)

On the basis of the similar molecular and cell damage, the effects of radiation and chemicals may be compared. Table 2.7 presents some data on chemicals which are present as the combustion products of various substances in urban atmospheric air. The last column contains the radiation doses, which produce the same biological

Table 2.7

Comparison of the hazardous effects of some combustion products and ionizing radiation

Compound	Concentration	Biological effect studied	Dose equivalent per week (mSv)
Ethylene	0.05 mm^3/l	mutation in mice	≈ 0.1
Ethylene	1 packet of cigarettes daily	mutation in mice	≈ 0.7
Formaldehyde	2×10^{-2} $\mu g/l$	cell killing in *in vitro* culture	≈ 0.5
Benzopyrene	2×10^{-3} $\mu g/l$	cell mutation in *in vitro* culture	≈ 0.06

effects as the chemicals in the given concentration during *an exposure of one week*. The Table demonstrates that the dose equivalent relating to even a single combustion product is higher than that due to the background radiation; moreover, some of the values roughly attain the dose equivalent limit at occupational exposure (cf. the preceding point 3).

Chemicals are usually tested first *in vitro* to reveal possible lethal mutations in some virus strain. In order to obtain a simple quantitative characterization of the effect, the probability of injury development in the initial stage of the incubation ($t=0$) is determined, and related to unit time and unit concentration. This quantity shows differences of several orders of magnitude for various compounds.

2.20. Therapeutic radiation sources

The biological effects produced by radiation may also be used for *therapeutic purposes*, especially in tumour therapy. The basis of this therapy was the recognition that rapidly dividing cells with simple (undifferentiated) structure are more sensitive to radiation than are strongly differentiated cells undergoing slow division. From this aspect healthy cells too are of different types, but tumour cells usually belong to the former type and can be destroyed without any substantial radiation injury of the healthy cells.

For therapeutic purposes, X-radiation, γ-radiation, accelerated electrons and β-radiation are used. Extensive and promising research work is being carried out on the applications of *neutrons, protons, heavy ions* and *pions*.

Teletherapy. Earlier, only the γ-radiation of the decay products of radium was utilized, and because of the long half-life of radium the equipment *(radium guns)* is still in use. Since the appearance of the artificial radioisotopes, mainly $^{60}_{27}Co$ and more rarely $^{137}_{55}Cs$, which are relatively simple and cheap to produce, *cobalt and caesium guns* have become widespread (Fig. 2.61, Supplement).

The most important radiophysical data of the used radiation sources are presented in Table 2.8. The radiosources emit γ-photons of various energies, but the Table gives

Table 2.8

Radiation data of γ-gun charges

Radiation source	Half-life (year)	Effective photon energy (MeV)
Radium series	1600	0.75
Cobalt-60	5.27	1.25
Caesium-137	30.1	0.662

only the effective photon energies, which are usually a sufficient guide with respect to their use. Each radiosource also emits β-radiation (the radium series contains α-radiating members too), but this is stopped by the wall of the irradiation head containing the radiosource, which results in the emission of X-rays and secondary electron radiation. In the preparation of a careful irradiation plan these types of radiation too must be taken into consideration.

The advantage of γ-radiation sources over X-ray tubes is that they emit harder photons of definite energy (line spectrum), which permits an increase of the relative depth dose[8] and a simultaneous decrease of the lateral scattering. The operation of γ-sources is simple and independent of the electric power supply. The load of γ-guns ranges between TBq and PBq.

The tissues to be treated are situated at a distance of 20–80 cm or more from the radiosource. This is the reason for the high load since the source must deposit a sufficient dose at this distance.

If the distance *RS* between the radiosource and the skin is increased, the absolute value of the dose decreases on the body surface and within the body, but the percentage depth dose inrceases. This is understandable by reference to Fig. 2.62. The distance *RS* in one case is 10 cm, and in the other 20 cm, while in both cases the centre *P* under treatment lies 10 cm below the skin. If, for the sake of simplicity, the absorption is disregarded, and it is considered that the intensity of radiation for a point-like source decreases with the square of the distance, it is easy to see that in the first case the depth dose is 25% of the skin dose, whereas in the second case it is 44.4%.

The spreading use of high-energy photons is justified by the very favourable dose distribution produced in many cases. The effective range of photoelectrons, Compton electrons and electron–po-

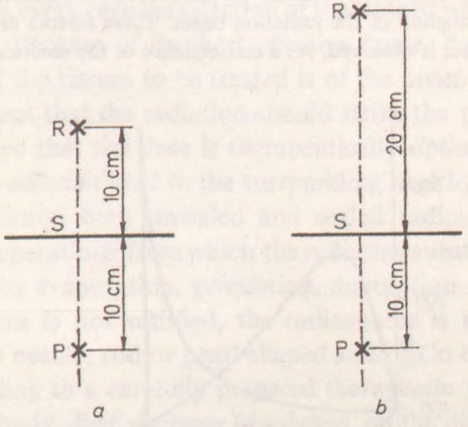

Fig. 2.62. Diagram relating to the increase of the relative depth dose

[8] The relative depth dose is defined as the ratio of the dose taken up by the focus at a certain depth below the skin to the dose measured on the surface of the body (skin dose), usually expressed as a percentage.

sitron pairs (together called secondary electrons) produced by sufficiently hard X- or γ-radiation is several mm, or even a few cm in the tissues. In such cases the dose distribution may be influenced substantially not only by the photon scattering, but also by *the scattering of the secondary electrons.* Figure 2.63 illustrates the situation arising close to the air–skin boundary. The dashed lines indicate the boundaries of the photon beam, the curved lines are the secondary electron tracks along which they cause ionization, and the points denote the sites of emission of secondary electrons. The density

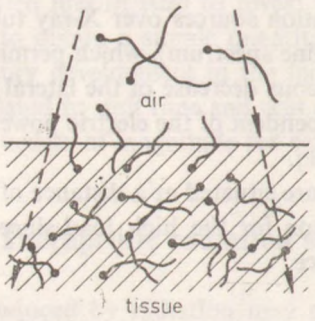

Fig. 2.63. Scattering of secondary electrons in the vicinity of a boundary surface

of these sites is higher in the tissues than in the air, and as a result more electrons pass from the tissues into the air than in the opposite direction. (A similar phenomenon may naturally occur wherever media absorbing X-radiation or γ-radiation to different extents are in contact with each other, for instance on the boundary surfaces between the bones and the soft tissues.)

The dose distribution in the tissues is demonstrated in Fig. 2.64 on actual examples. The abscissa gives the depth of penetration measured from the surface of the body, and the ordinate the percentage depth dose. Curve *a* relates to 0.2 MeV photons, and curve *b* to 22 MeV photons. In the first case the dose decreases monotonously with the distance from the surface, which is a consequence of the divergence and extinction of the radiation beam. These factors are also effective in case *b* but here an additional effect is observed. As a consequence of the secondary electron scattering, on

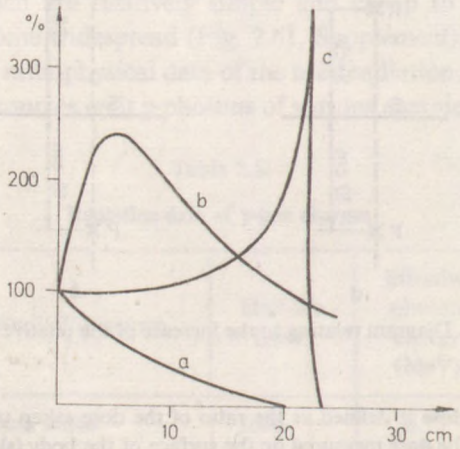

Fig. 2.64. Relative depth dose as a function of depth

progressing from the surface towards the tissues deeper below the skin the dose first increases, and the decrease becomes predominant only later. Instead of a monotonously decreasing function, the curve obtained exhibits a *maximum*. It may readily be seen that the peak in the curve appears the later, the longer the stopping path length of the secondary electrons, i.e. the harder the radiation. With harder radiation the shift of the maximum towards greater depths is further enhanced by the fact that the secondary electrons are increasingly scattered in the forward direction, i.e. in the direction of the incident beam. (This asymmetry is not shown in Fig. 2.64.) For instance, with 22 MeV photons the maximum lies at a depth of 4–5 cm, and with 35 MeV photons at a depth of 6–7 cm below the surface. In principle the formation of a maximum is also to be expected with softer radiation, but it is so close to the surface that it practically coincides with it. By a proper selection of the hardness of the radiation, the dose maximum can be obtained at the focus to be destroyed, and this receives a sufficiently high dose without injury to the healthy tissues close to the surface.

Similar phenomena also occur with *high-energy electrons*. In this case too the dose maximum shifts towards the deeper tissues, the higher the energy of the radiation applied. With fast electrons, however, after the maximum is attained the decrease is faster than in the case of photon irradiation. In some therapeutic applications, therefore, accelerated electrons are used instead of photons, in order to protect the deeper tissues.

This latter advantage is even more marked with *heavier charged particles*, e.g. with monochromatic proton radiation (curve *c* in Fig. 2.64). This is connected with the rapid increase in the ionizing power towards the end of the path of the protons, followed by a sharp decrease (see Fig. 2.33).

The above dose distribution types may also be observed if fast neutrons are applied. The outlined characteristics of the dose distribution are due to the scattering of the protons. The position of the dose maximum in the tissues lies the deeper, the higher the energy of the neutrons used for irradiation, i.e. the higher the energy of the protons inducing the ionization.

Contact methods. For irradiation, the radiosource is either fastened to the surface of the body, or introduced into the pathologic tissue or into the natural or pathologic cavities (close to the pathologic tissues) of the organism. The great variety of artificial radioisotopes allows in every case the selection of the isotope with the most favourable radiation parameters. Because of its short effective range, β-radiation is used only when the thickness of the tissues to be treated is of the order of at most a few mm. It is a basic requirement that the radiation should strike the pathologic tissues in a uniform *distribution*, so that the dose is therapeutically optimum and at the same time the *unnecessary radiation* load to the surrounding healthy tissues is avoided.

For contact irradiation both unsealed and sealed radiosources are used. The *sealed* sources are preparations from which the radiating substance, if applied properly, cannot escape by evaporation, powdering, dissolution or abrasion. If merely one of these conditions is not satisfied, the radiosource is unsealed. Examples of sealed sources are the needle, rod or pearl-shaped solid $^{60}_{27}Co$ or $^{137}_{55}Cs$ sources, which are positioned according to a carefully prepared therapeutic plan on the surface or in the cavities of the body. *Radioisotopes in solution*, on the other hand, are unsealed sources: for instance, a colloidal solution of $^{198}_{79}Au$, which is administered by infiltration to the tumour (in the event of an appropriate colloidal grain size, the $^{198}_{79}Au$ remains at the site of infiltration).

Of the natural isotopes, the members of the *radium series* are administered in the form of sealed radiosources. In the most frequently applied procedure a certain

quantity of some radium salt is enclosed in a needle or rod-shaped platinum or gold case.

After the sealing all of the decay products (including lead) appear within the case. The quantities of the individual radioactive products first increase for some time, but since they simultaneously undergo disintegration, dynamic equilibrium (radioactive equilibrium) is soon attained: each derivative disintegrates at the same rate as it is formed. Radium attains equilibrium with its radioactive derivatives in roughly one month. As radium has a long half-life, the equilibrium remains unchanged for years (its activity decreases by only 0.044% annually), which means that the radiosource radiates with practically unchanged intensity.

The wall of the case is generally thick enough to absorb the α-, β- and even the very soft γ-radiation of the individual components of the series. Thus, only the harder radiation of ^{214}Pb (RaB) and ^{214}Bi (RaC), produced by the decay of the intermediate products, is used, and not that of radium after which the preparation is named. The γ-radiation of the radium preparation consists of lines of different hardness, but as concerns its absorption by the tissues it virtually behaves as if it consisted only of 0.75 MeV radiation. One of the radium derivatives, radon, is gaseous. Consequently, if the case seals poorly, the gas will escape and decay further in the open air. The sealed preparation then becomes an unsealed one.

The use of radioactive preparations is regulated by *radiation-protection* rules, which must be strictly observed at all times.

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3. MICROSCOPIC AND SUBMICROSCOPIC METHODS IN BIOLOGICAL STRUCTURE ANALYSIS

A common feature of the methods of structure analysis is that the specimen is subjected to some effect and the consequences are subsequently investigated. The analysis can be extended to changes occurring in the agent, the sample, or both. The possible agents include thermal effects, magnetic, electric or other fields, and the sample is often exposed to a radiation or particle beam.

The latter technique of structure analysis is a particularly wide-ranging one, since various radiation and particle types can be used, and numerous interactions with the sample may be produced. Examples are illumination with light or irradiation with X-rays, electrons, ions or atoms, where the interactions may result in scattering, diffraction or absorption, or the emission of radiation or a particle different from the incident one. These methods include studies with the light microscope based on light scattering, which have a long tradition in biology and in medicine, while a more recent application is the study of the energy spectrum of electrons produced in the sample by X-ray irradiation.

The methods of structure analysis are undergoing continuous development and new techniques are constantly emerging. A variety of possibilities is of great advantage, for the different methods reveal different properties of the system under investigation, and provide mutually complementary information.

Structure analysis is dealt with in other chapters of this book too; for instance, the nuclear methods have been treated in Chapter 2, and the use of ultrasound will be discussed in Chapter 5. These are macroscopic methods, and in the present chapter (as indicated by its title) we wish to outline the possibilities of revealing finer structural details (without any aim to completeness).

3.1. Light microscopes

3.1.1. Magnification

The observed objects (and their details) appear to be the larger, the greater the visual angle associated with them (Fig. 3.1). The *visual angle* (φ) is defined as the angle enclosed by the rays arriving from the outermost points of the object and traversing

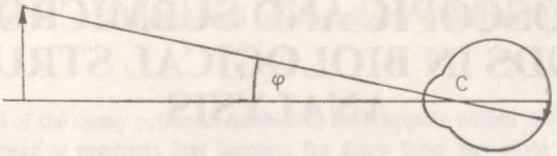


Fig. 3.1. Visual angle

the central nodal point (C) of the eye. The smallest visual angle at which two object points can still be distinguished with the naked eye (without any special aid) has been found to be 1 angular minute. If the visual angle of two points is smaller than this, they cannot be resolved by the eye, which in this case sees only one point instead of two. If the object is at the distance of clear sight (25 cm), two points can be distinguished only if they are at least 70 μm apart, since they then subtend an angle of 1 angular minute from 25 cm. With simple magnifying glasses the dimensions that can be observed may be reduced by one order of magnitude, while with the light microscope the reduction is of several orders.

The essential parts of the microscope are as follows:

(a) the illuminating system, which projects the light through a convex lens system, the *condenser,* onto the object;

(b) the *objective,* a convex lens system directed onto the object;

(c) the *eyepiece,* another convex lens system, through which the produced image is observed. The image formation is demonstrated in Fig. 3.2. For simplicity the exact construction of the image is omitted, and the lens systems are depicted as thin lenses. F_1 and F_1' are the focal points of the objective, while F_2 and F_2' are those of the eyepiece. The distance between the points F_1' and F_2 is usually called the optical tube length and is denoted by d. The objective is brought nearer to the object (AA') so that it is situated outside but close to the focal point F_1. In this way the objective produces from the object a magnified, reversed image (BB') on the opposite side and at a large distance relative to the focal length. This image is viewed through the eyepiece, which acts as a simple magnifying glass. The image produced by the objective lies within the focal length of the eyepiece, close to the focal point F_2. From the first image of the objective the eyepiece produces a virtual, magnified and erect image (CC'). Thus, *in the microscope a virtual, magnified and reversed image of the object is observed.*

The magnification of a single lens is equal to the ratio of the image and object distances. Consequently, the linear magnification of the microscope can be estimated in the following way. In the image formation of the objective the object distance is nearly equal to the focal length f_1 and the image distance is nearly equal to the optical tube length d of the microscope, which gives the magnification N_1 of the objective as d/f_1. In the image formation of the eyepiece the object distance is approximately equal to the focal length f_2 of the eyepiece, and the image distance is $-a$, where a (=25 cm) is the distance of clear sight. (The negative sign indicates that the virtual

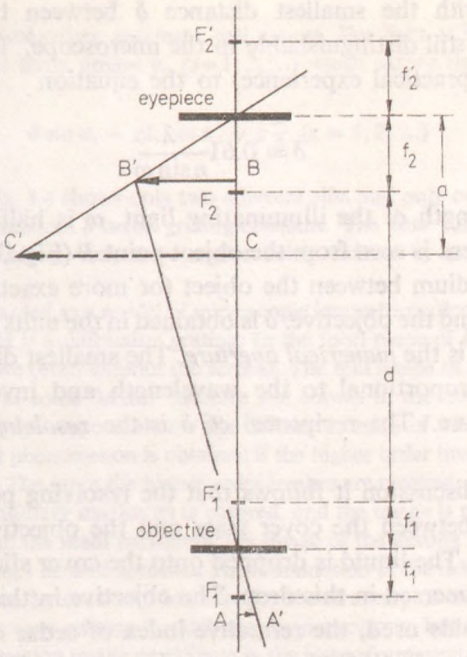


Fig. 3.2. Image formation in the microscope

mage CC' is on the same side of the eyepiece as BB'.) It follows that the magnification N_2 of the eyepiece is equal to $-a/f_2$. Thus, the total magnification is

$$N = N_1 N_2 = -\frac{da}{f_1 f_2} \qquad [3.1]$$

The magnification of the microscope (as an absolute value) will be the higher, the smaller the focal lengths of the objective and the eyepiece.

3.1.2. Resolving power

The microscope reveals many fine details of the object, which could not be observed with the naked eye. However, even with properly corrected lens systems of small focal lengths, the resolution of small details is limited by the wave nature of light. The problem cannot be solved with the use of more lens systems, since the details of the object are revealed by the objective lens. The eyepiece does not add new detail, but only magnifies the image produced by the objective to ensure good observation of the already revealed details. This task is satisfactorily achieved by the eyepiece without too large a magnification. The use of further lens systems adds no new information and is consequently superfluous.

We next deal with the smallest distance δ between two object details (also called object points) still distinguishable in the microscope. The considerations lead (in agreement with practical experience) to the equation

$$\delta = 0.61\frac{\lambda}{n \sin \omega} \qquad [3.2]$$

Here λ is the wavelength of the illuminating light, ω is half the *aperture angle*, in which the objective lens is seen from the object point P (Fig. 3.3), and n is the refractive index of the medium between the object (or more exactly the thin cover glass covering the object) and the objective. δ is obtained in the units in which λ is measured. The quantity $n \sin \omega$ is the *numerical aperture*. The smallest distance still resolved by the microscope is proportional to the wavelength and inversely proportional to the numerical aperture. The reciprocal of δ is the *resolving power* of the microscope.

From the above discussion it follows that the resolving power can be increased by filling the space between the cover glass and the objective lens with a liquid of high refractive index. The liquid is dropped onto the cover slide, and the frontal lens of the objective is immersed in this drop. The objective in this case is the *immersion objective*. Of the liquids used, the refractive index of cedar oil is 1.51, and that of monobromonaphthalene is 1.66. Since the maximum value of ω in practice is about 70° ($\sin 70 \approx 0.95$), the smallest distance still resolved for visible light ($\lambda = 450$ nm) is approximately 200 nm.

[3.2] can be obtained from the diffraction of light. Consider Fig. 3.4*a*, which demonstrates the diffraction of light by a diffraction grating. The slit-shaped light source S is perpendicular to the plane of the drawing. The rays from the light source pass as a parallel beam through the lens L_1 and arrive at the lens L_2, which projects the image of the slit onto the screen E situated in its focal plane. If a diffraction grating (G) is placed between the two lenses (the slits of the grating are parallel to the slit-shaped light source), the image will be multiplied as a result of diffraction: on both sides of the original central image (also called *zeroth order image*, or *main maximum*) first, second, etc.

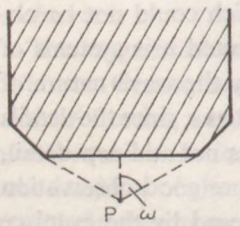


Fig. 3.3. Aperture angle of the objective (2ω)

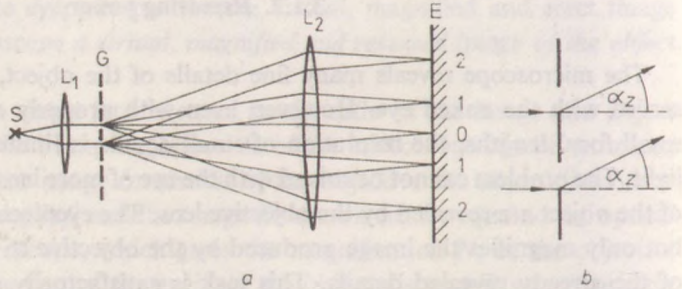


Fig. 3.4. Diagrams relating to the resolving power of the microscope

order diffraction images *(subsidiary maxima)* will appear. For light of wavelength λ these latter images are formed only at those angles α_z ($z=1, 2, \ldots$) which satisfy the equation

$$\delta \sin \alpha_z = z\lambda, \quad \sin \alpha_z = z\frac{\alpha}{\delta} \quad (z = 1, 2, \ldots) \qquad [3.3]$$

For illustration of [3.3], Fig. 3.4 shows only two adjacent slits and only one pair of diffracted rays propagating in the same direction. δ is the grating constant. The first subsidiary maximum is at the angle α_1, the second at α_2, etc., these angles being defined by the equations $\sin \alpha_1 = \lambda/\delta$, $\sin \alpha_2 = 2\lambda/\delta$, etc.

Figure 3.4*a* may be regarded as a model of microscopic image formation. L_1 is the condenser and L_2 the objective. The object is a diffraction grating. In the focal plane of L_2 a diffraction pattern is produced as described above (even without the screen). The real image of the object (in the present case the grating) appears at some further distance not shown in the diagram. The image of the object is produced by the rays responsible for the diffracted image of the light source in the focal plane of L_2. An interesting phenomenon is obtained if the higher order images are covered by a diaphragm in the focal plane. The more the higher order images are covered, the less perfect the image of the grating. If every subsidiary maximum is covered, and the image is produced only by the primary beam passing through the main maximum, the image of the grating becomes unrecognizable: the lines of the grating cannot be distinguished. The illumination of the field of sight is uniform, and no details of the grating can be observed. In order for the structure of the grating to be seen, it is necessary that not only the rays passing through the main maximum but also the rays propagating through the first order diffraction image participate in the image formation. However, this condition is satisfied only if the grating constant (δ) is not too small. This can easily be understood, because in the case of a too small δ the rays arriving from the grating and propagating towards the first order ($z=1$) diffraction image would enclose such a large angle with the lens axis that they would not fall on the lens. It follows that the angle α_1 associated with the first order diffraction image must not be larger than the half aperture angle ω of the objective; its maximum value is $\alpha_1 = \omega$, and hence $\sin \alpha_1 = \sin \omega$. For a given ω and λ, the lines of the grating become visible only for the grating constant δ which satisfies the equation $\delta = \lambda/\sin \omega$. With more exact reasoning, δ is given by $\delta = 0.61\lambda/\sin \omega$. It has so far been assumed that the space between the object and objective is filled with air, and λ indicates the wavelength in air. In a medium with refractive index n the wavelength will be λ/n. Consequently, if there is a medium of refractive index n between the object and the objective, λ/n should be used instead of λ, which leads to [3.2].

3.1.3. Special light microscopes

1. Ultraviolet microscope. According to [3.2] the resolution of the microscope can be improved if ultraviolet light of shorter wavelength is used instead of visible light. However, with this method quartz lenses must be applied, since glass absorbs ultraviolet radiation. The image is not visible with the naked eye; if the eyepiece is raised slightly, a real image is produced and can be displayed on a luminescent screen or a photographic plate.

2. Ultramicroscope. With the usual illumination the object details appear as more or less dark domains in the otherwise illuminated visual field. However, if the object is illuminated so that only the rays diffracted by the details of the object can enter the

objective (which is equivalent with the covering of the main maximum), the boundary lines of the object details become visible as bright spots on a dark background. Figure 3.5 (see Supplement) shows photographs of the same biological object taken with a normal light microscope (*a*) and with an ultramicroscope (*b*). In the ultramicroscope, particles become visible whose size is below the resolving power. These small particles shine against the dark background like stars in the nocturnal sky. Details are not visible; only their presence, position and motion can be observed. The conditions discussed above for the resolving power of the microscope hold here too for the distinction of two such particles. The particle is the better discerned, the more its refractive index differs from that of the environment. For instance, the conditions for the observation of protein particles are less favourable than those for metal colloids. In this latter case even particles 10 nm in size can be observed well; the name ultramicroscope refers to these favourable conditions. Particles smaller than 5 nm *(amicroscopic particles)*, however, cannot be observed with this method.

3. 3D condenser. The quality of the microscopic image (within the limits of resolution) is strongly influenced by the illumination of the object. The observations mentioned in the previous point relate to this fact, and the principles of the *3D* condenser too are based on it. With the aid of this condenser the axial illumination is combined with lateral illumination. This system results in increases in the plasticity and contrast of the image. The notation *3D* refers to the three-dimensional character of the image obtained.

4. Phase contrast microscope. The observation of certain properties of various objects is made possible by phase contrast microscopy. The eye can distinguish only details which differ from each other either in illumination or in colour. This is clearly due to the details of the object absorbing the illuminating light in different ways. However, the object may have properties which do not give any contrast of illumination or colour; for instance, the various parts of the object may differ in thickness or refractive index and otherwise transmit the light with almost no absorption. The phase contrast microscope allows observation of these properties by converting them into differences of illumination (Fig. 3.5*c*). Without going into details, we shall mention only that for this purpose a disc or ring-like small transparent plate of suitable thickness and a diameter of a few mm, the *phase plate,* is placed in the focal plane of the objective, and the illumination is modified accordingly. Every microscope can also be used as a phase contrast microscope if its usual objective is replaced by an objective provided with a phase plate.

5. Polarization microscope. The microscope constructed for the study of birefringence contains not only the lens systems of the ordinary microscope but also two *Nicol prisms (nicols) or polaroid plates.* One of the prisms (or plates) operates as a polarizer and the other as an analyzer. The polarizer is situated below the condenser lens, and the analyzer above the objective. The analyzer can be rotated around the light beam as axis. In this way its plane of polarization can be changed from the parallel

to the crossed position relative to the polarizer. The object stage, provided with an angular scale, can also be rotated. The examination is carried out by rotation of the nicols into the crossed position prior to insertion of the object, the field of view thereby becoming dark. On insertion of the object and rotation of the stage, the birefringent details of the object become bright in certain positions and then darken on further rotation.

6. Luminescence microscope. Luminescence can be used in microscopic examinations, because most organic compounds, including those found in the living organism, emit visible luminescent light characteristic of their chemical structure when illuminated with ultraviolet light. The different compounds generally display different colours when luminescing, and thus the various cells or cellular components with different chemical compositions can be well distinguished in the section. Besides the intrinsic luminescence, another phenomenon too can be used: cells and tissues adsorb the luminescent dye from extremely dilute (1:1,000–1:5,000,000) aqueous solutions of these compounds *(fluorochromes)*, and the adsorbed dyes emit luminescent light of various colours, depending upon the cell or tissue type. The dilute solution does not affect the structure or functions of living cells.

The great advantage of both luminescent methods over the classical microstaining procedures is the avoidance of the rough chemical effects of fixing and staining and of the possible change of the living tissues. A further advantage is the possibility to examine biopsy samples within a few hours. The method can also be used to reveal the presence of acid-resistant bacteria.

The construction of the luminescence microscope is very similar to that of the ordinary microscope, the only difference being that the condenser and the slides are made of special glass transmitting ultraviolet light; further, usually in the frontal piece of the objective lens system, a filter is used which filters out the ultraviolet light transmitted by the object.

The object is normally *illuminated* by a mercury vapour lamp, or an arc lamp with metal electrodes. The visible light of these lamps is filtered out to prevent interaction with the luminescent light.

We have so far spoken merely of ultraviolet illumination, without regard to its spectral composition. However, it is a well-known effect that ultraviolet (and sometimes also visible) light of various frequencies may excite different domains of the object. Consequently, with the application of suitable filters, transmitting the exciting light only in a narrow frequency band, additional fine details may be distinguished. The possibilities are even wider, for the luminescence can be excited not only by ultraviolet light, but also by short wavelength visible light.

7. Binocular microscopes. Microscopic observation is more convenient and less tiring for the eye if both eyes can be used. This may be achieved with the binocular microscope, which has only one objective but two eyepieces. The rays passing through

the objective are separated into two beams by a semitransparent and semireflecting prism before the formation of the real image. Each of the separated beams produces an image which can be observed with the two eyepieces. The distance between the eyepieces can be adjusted to accord to the interocular distance.

8. Stereomicroscope. Three-dimensional images are obtained with the stereomicroscope, which contains two objectives and two eyepieces. This microscope consists essentially of two microscopes built together. One of the microscopes forms the image of the object from a little to its left, and the other one from its right side, in this way two slightly different images being obtained. If one of these images is viewed with one eye and the other image with the other eye, a realistic three-dimensional image is seen. Stereomicroscopes can be used only at low magnification (at most 100×), for at higher magnification the depth of focus is small and stereoscopic observation becomes impossible.

3.2. Electron microscopes

In the electron microscope, electrons accelerated in an electric field are used instead of light for image production. In the light microscope the light rays are directed by refraction, whereas in the electron microscope the path of the electrons is influenced by electric or magnetic fields.

3.2.1. Electron lenses

The main parts of the electron microscope are the electric and magnetic lenses jointly referred to as *electron lenses*. Electric and magnetic lenses are electric and magnetic fields focusing at one point the electrons arriving from one point.

Electric lenses are produced in practice with three electrodes of high-accuracy cylindrical symmetry (Fig. 3.6). A voltage of several ten thousand volts is applied

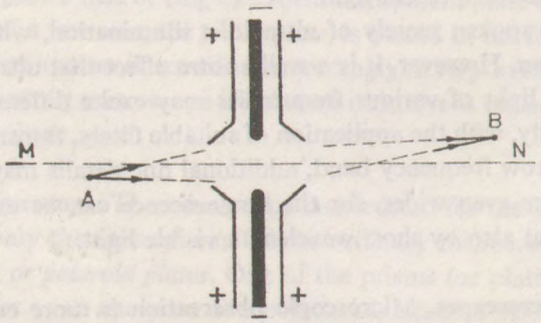


Fig. 3.6. Schematic drawing of an electric lens
The straight line *MN* indicates the lens axis; the electron beam arriving from the object point *A* is focused at *B*

to these electrodes in such a way that the central electrode is connected with the negative, and the other two electrodes with the positive pole of the voltage source (or conversely). The electron beam passes through a circular slit, and the lens effect is produced by the field of cylindrical symmetry developing in the slit and its surroundings. The focal length of the lens is the shorter, the higher the field strength in the smallest possible space.

Magnetic lenses are coils in which an electric current flows (Fig. 3.7). The electrons travel through a circular slit surrounded by the coil. The coil is covered by an iron coating (*I*) with a gap in its inner side. This arrangement allows the production of a strong magnetic field of cylindrical symmetry within a small space. The focal length can be decreased by inserting iron pole pieces (*P*) into the gap, since this increases the field strength.

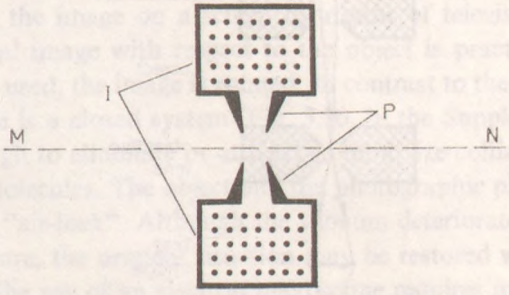


Fig. 3.7. Schematic drawing of a magnetic lens
The black points indicate the intercept of the coil windings with the plane of the drawing

Besides these factors, the focal length also depends upon the electron velocity. A smaller velocity results in a smaller focal length, and vice versa. The accelerating voltage is usually several ten thousand volts, and in this case the focal length is a few mm.

The relations of image formation used in light optics hold for electron lenses too. The aberrations of image formation are similar in the two cases. Even chromatic aberration has its electron optical counterpart, and becomes observable if the velocity of the electrons is not homogeneous. With magnetic lenses a further aberration is due to the spiral path of the electrons in the magnetic field. Thus the image is usually rotated with respect to the object around the lens axis. The abberrations for electron lenses can be decreased only by applying a monochromatic electron beam enclosing a small angle with the lens axis.

3.2.2. Construction and resolving power

The construction of the electron microscope is similar to that of the light microscope (Fig. 3.8). The light source is substituted by an *electron source,* which is either a hot filament or a cold electron emitter. The electron beam leaving the source and accelerated is focused by the electron lens (condenser) onto the object. The electrons are scattered to various extents by the details of the object (Fig. 3.9).

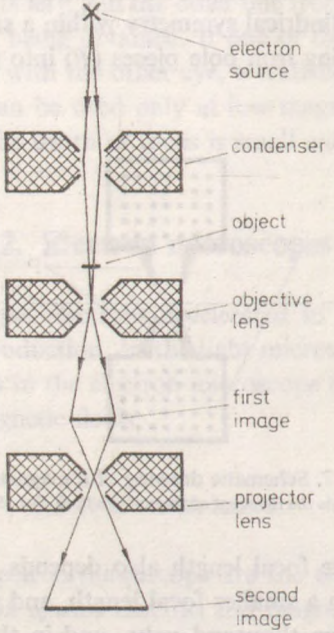


Fig. 3.8. Outline of an electron microscope constructed from magnetic lenses

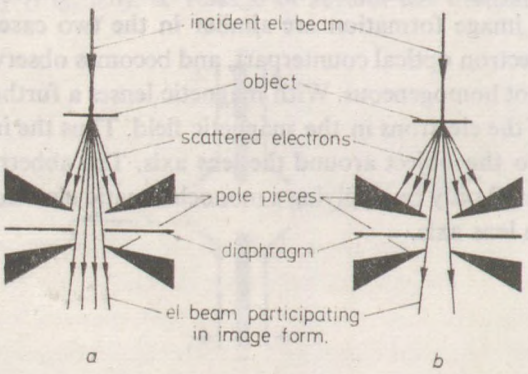


Fig. 3.9. The role of scattering in the resolution of the details; the electron beam for a weakly scattering detail (*a*) and a strongly scattering detail (*b*)

Some of the scattered electrons are arrested by a circular aperture. Fewer electrons are transmitted by the aperture from the details scattering more strongly, and a greater number from those scattering more weakly. After the aperture the electron beam passes the objective lens, which focuses in one point the divergent beam arriving from the object and, depending on the adjusted object distance, produces a real and magnified image of the object. The image can be made visible on a luminescent screen or a photographic plate. Lighter or darker spots are produced, depending upon the stronger or weaker scattering of the electrons on the object points. In this way well contrasted images are obtained, the details of the object being observable with the human eye. However, a screen is placed in the image plane of the objective only rarely, for control purposes, and the image is magnified further by the projector lens, which from the first image produces a magnified, real image on a luminescent screen or photographic plate. In more recently developed electron microscopes it is possible to display the image on a screen by means of television techniques. The position of this final image with respect to the object is practically immaterial; if magnetic lenses are used, the image is rotated. In contrast to the light microscope the electron microscope is a closed system (Fig. 3.10, in the Supplement). It requires a vacuum good enough to eliminate or at least to minimize collisions of the electrons with air or other molecules. The object and the photographic plate are placed in the microscope via an "air-lock". Although the vacuum deteriorates by a few millibars during this procedure, the original situation may be restored with a vacuum pump in some minutes. The use of an electron microscope requires much care, and for its operation the continuous, undisturbed functioning of several subsidiary devices is necessary. For instance, the acceleration of the electrons and the operation of the electro-optical lenses require special electric equipment to produce the suitable high voltage. Special care must be taken with regard to stabilization of the voltage.

With the electron microscopes generally used, object details approximately a hundred times smaller can be resolved than with light microscopes (the smallest distance which can be resolved at present is about 0.5 nm). Consequently, the *resolving power* of an electron microscope is about a hundred times higher than of light microscopes. With special electron microscopes a resolution even several times higher has been attained. In order to make the resulting resolved details observable with the naked eye, the image projected onto a luminescent screen or photoplate is magnified further about ten times by light optical means.

For the resolving power of an electron microscope, a relation similar to that for the light microscope holds, but the light wavelength must be substituted by the matter wavelength of the electrons (cf. section 1.1). This wavelength is inversely proportional to the velocity of the electrons and at an accelerating voltage of about 50 kV is roughly five orders of magnitude smaller than the wavelength of visible light. An increase by five orders of magnitude in the resolving power would therefore also be expected. Though this is in principle true, in practice we have to be satisfied with the 100-fold increase: in order to decrease aberrations we have to work

with electron beams of small angular aperture, i.e. with a small numerical aperture. The increase of the resolving power is likewise hampered by the difficulties involved in the preparation of sufficiently thin and undistorted sections, that is by the fact that it is difficult to avoid damage to the object during other stages of sample preparation.

3.2.3. Special procedures

1. Scanning electron microscope. In this configuration the object is scanned by a well-focused electron beam, in the same way as the electron beam moves in the video tube. The details of the object reflect the electrons to different extents. The equipment produces electric signals proportional to the reflected electron currents, and these signals modulate the intensity of the electron beam of a television monitor. The electron beam of the monitor and the scanning beam are sweeping synchronously producing the image of the object on the screen. Figure 3.11*a* in the Supplement shows an electron microgram recorded with a traditional electron microscope, while in Fig. 3.11*b–c* a picture obtained with scanning electron microscope can be seen.

Besides the above-discussed surface screening the scanning method is also used in transmission mode. In this case the resolving power is weaker than at fixed illumination but a greater area of the object can be imaged.

2. X-ray microanalysis. Most of the electron microscopes are also suitable for energy dispersive X-ray microanalysis. Its main point is that, by bombardment of the sample with focused electron beam, a characteristic X-ray is produced. From the X-ray spectrum the chemical composition and the amount of the components of the sample can be determined. Fixed and scanning modes of operation are available in this case, too. By using the scanning mode the local changes in the composition of the sample can also be revealed.

3.3. Optical spectrometry

The transition permitted by the selection rules between two possible states of atomic systems (atoms, molecules, solids, liquids) may be achieved either by photon uptake (absorption) or by photon release (emission). The energy of the absorbed or emitted photons is equal to the difference between the energies for the two states. These energies may be determined from spectral studies. Information can be obtained on the electronic transitions, and the vibrational and rotational states. Moreover, in an indirect way spectrometry yields still further information, e.g. the following data can be determined:

(a) the geometric positions (distance and directions) of the atoms or atom groups within the molecules;

(b) the bond strengths between the atoms and atom groups, and the bond types (ionic, covalent bonds, etc.);

(c) the conditions of molecular dissociation, and the energies of dissociation in the ground and the excited states;

(d) conclusions can be drawn about the changes occurring in the molecular configurations in response to environmental changes.

The changes in the electron energies are of the order of magnitude of an electron volt; the vibrational energy differences are lower by one order of magnitude and the rotational energies are a further order smaller. The electron energies are in the visible and ultraviolet, and the vibrational energies in the infrared range (Fig. 3.12). The rotational energies are comparable with the energy quanta of the far infrared, the electromagnetic microwaves and the radio waves.

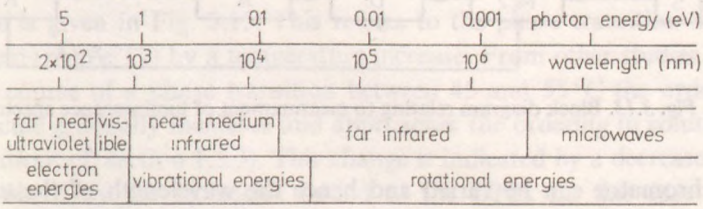


Fig. 3.12. Quantized energy types of molecules, wavelength and photon energy ranges of the spectra corresponding to the energy transitions

3.3.1. Emission spectrometry

The *emission spectrum* is the distribution of the emitted light intensity as a function of wavelength (frequency, photon energy). The spectrum is obtained after excitation of the sample, for instance by heat or electric energy (flame colouring, arc or spark discharge). Optical excitation is frequently applied; this is the *photoluminescent method.* In the event of thermal or electric excitation the substances dissociate into their atoms or ions, such excitation therefore resulting in atomic or ionic spectra. Molecules can be excited only optically by irradiating them in their optical absorption band (cf. section 3.3.2). The energy absorbed on excitation is not always released in the form of an emitted photon, for the molecule may return to its ground state in a radiationless transition. Nucleotide bases at room temperature behave in the latter way, whereas aromatic amino acids emit a greater part of the absorbed energy.

In photoluminescence two kinds of spectra may be obtained. One is the *emission spectrum,* obtained when exciting light of a given wavelength is used and the spectrum of the resulting luminescent light is determined. The other type is the *excitation spectrum,* obtained when the light intensity emitted at a given wavelength is recorded as a function of the wavelength of the exciting light.

For the production and study of both types of spectra the apparatus outlined in Fig. 3.13 is used. It is described as used for measurement of the emission spectrum, but it can equally be applied to produce excitation spectra. Excitation is usually performed with a light source (*L*) emitting in the UV and visible range, provided with a colour selective filter or a monochromator (*Mc1*). The monochromator resolves the incident light into its components by means of an optical prism or grating. On rotation of the resolving elements, the wavelength of the light falling on the exit slit of

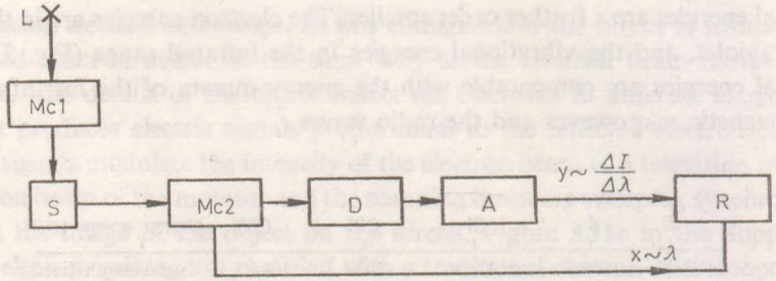


Fig. 3.13. Block diagram relating to measurement of luminescence spectra

the monochromator can be varied and hence the wavelength of the exciting light can be changed. The light emitted by the sample (*S*) in response to the excitation is analysed by a second monochromator (*Mc2*). Rotation of the resolving element of *Mc2* causes different wavelength ranges of the studied luminescence spectrum to fall on the exit slit and thus the whole emission spectrum can be obtained. The spectral bandwidth ($\Delta\lambda$) of the light emerging through the slit can be varied to some degree, since it depends on the optical parameters of the resolving element (e.g. the dispersion of the prism, the grating constant of the grating) and on the width of the slit. The emerging light intensity (ΔI) (or more exactly $\Delta I/\Delta\lambda$) is converted by the detector (*D*; e.g. a photomultiplier) into an electric signal, which is amplified by an electronic amplifier unit (*A*). The amplified signal is recorded by an $X-Y$ recorder (*R*). *Mc2* and *R* are coupled in such a form that the wavelength is recorded on the X axis.

As an example, luminescence spectra of aromatic amino acids are shown in Fig. 3.14; these can be used for analytical purposes. The identification and simultaneous detection of the amino acids at a given hydrogen ion concentration, temperature, solvent, etc. can be achieved by recording the spectral positions of the emission bands.

The luminescence spectrum (not only the emitted light intensity but the degree of polarization too) may give information on the environment of the emitting molecule (cf. sections 1.3.1–1.3.3). As an example, mention may be made of molecules which can bind to the nucleic acid, or more exactly which may be intercalated between the base planes, having an emitted light intensity depending on the degree of ordering within the DNA chain. Thus, with the aid of intercalated luminescent molecules information may be obtained on the higher order structure of nucleic acids.

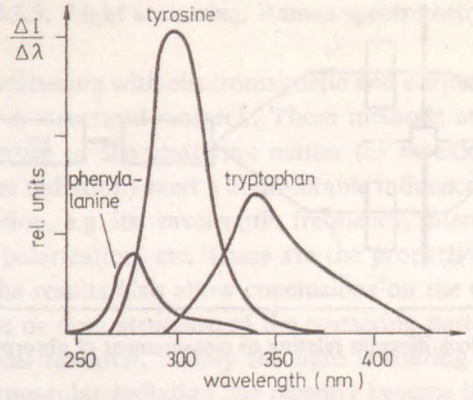


Fig. 3.14. Luminescence spectra of aromatic amino acids

An example is given in Fig. 3.15. This relates to the phase transition induced in a nucleoprotein (phage T7) by a temperature increase. From other studies it is known that in the course of a phase transition between 45 and 55 °C the ordering of the DNA molecule gradually increases and approaches the ordering in solution (regular *B* conformation; cf. section 1.5.3). This change is indicated by a decrease in the light intensity emitted by the molecules bound to the DNA (in this case proflavine).

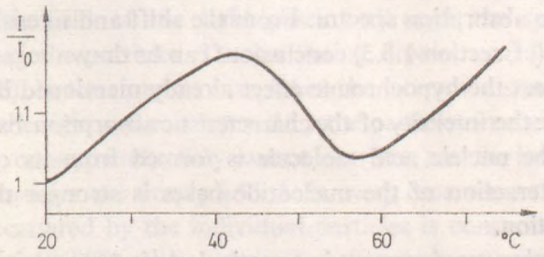


Fig. 3.15. Detection of the conformational rearrangement of the T7 phage-nucleoprotein with the aid of the light emitted (at 510 nm) by the proflavine molecules bound to the nucleic acid
The abscissa gives the temperature, and the ordinate the light intensity emitted at the studied temperature (I) relative to that emitted at room temperature (I_0)

3.3.2. Absorption spectrometry

The absorption spectrum is the distribution of the extinction $\log(I_0/I)$ (cf. section 2.3.1) as a function of wavelength. It is measured with the apparatus shown in Fig. 3.16. The continuous spectrum of a light source (L) is wavelength-resolved by a monochromator (Mc). The light selected by the exit slit and considered monochromatic is split into two beams. One beam passes through the investigated sample (S),

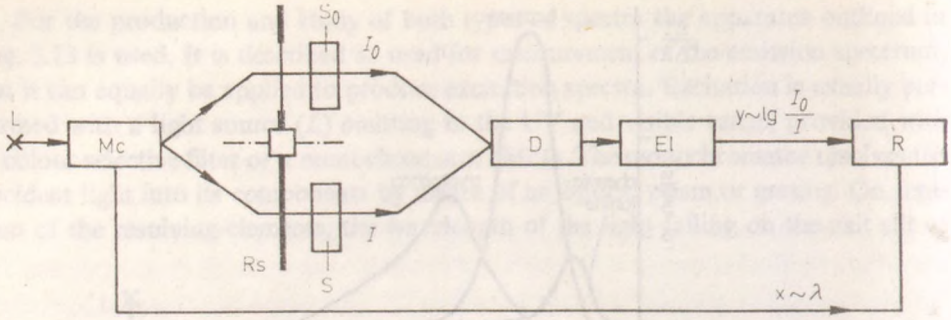


Fig. 3.16. Block diagram relating to measurement of absorption spectrum

and the other through the reference sample (S_0) used for comparison (see below). The rotating sector (R_s) lets the beams pass the samples alternately and thus the detector (D) records the intensities I and I_0 alternately. Log(I_0/I) is produced by an electronic system (El). The $X-Y$ recorder (R) plots the wavelength-dependent extinction. The use of the reference sample eliminates in a simple way the intensity losses due to reflexion and (in the case of solutions) to solvent and cuvette absorption.

As concerns biological structure analysis, the visible and ultraviolet absorption spectra relating to electron transitions are of the same importance as the *infrared* spectra relating to *vibrational* transitions. Certain atomic groups of the molecules give rise to characteristic light absorption. The existence of these *chromophores* can be detected by the absorption spectra. From the shift and intensity changes in their absorption bands (cf. section 1.5.3) conclusions can be drawn regarding their environment. In this respect the hypochromic effect already mentioned in the case of DNA may be referred to: the intensity of the characteristic absorption band (about 260 nm) decreases when the nucleic acid molecule is formed from its constituents, as the intramolecular interaction of the nucleotide bases is stronger than when they are "isolated" in solution.

Infrared absorption measurement is a particularly important method in biological and organic chemical structure analysis. By this means, many functional groups (carbonyl, hydroxy, amino, etc.) can be detected simply, even in relatively complex molecules, since the vibrational frequencies of these groups (determined by the strengths of the chemical bonds and the atomic weights of the vibrating atoms) depend only slightly upon the carbon chains coupled to the groups. From the small wavelength shifts in the absorption bands characteristic of the functional groups, information can be obtained about the structure of the surroundings of these groups.

3.3.3. Light scattering. Raman spectrometry

Studies involving scattering with electromagnetic and corpuscular radiation began several decades ago in structural research. These methods are based on the experience that the properties of the scattering matter (as revealed by the interactions of its particles with the radiation) exert a considerable influence on the characteristics of the scattered radiation, e.g. its wavelength, frequency, intensity, the spatial intensity distribution, the polarization, etc. These are the properties which are measured experimentally, but the results also allow conclusions on the dimensions, shape, internal density changes or even structure of the scattering particles.

In current biological research, mainly the light scattering methods are applied, though the use of corpuscular radiation has recently become important. The investigations usually yield information about the morphology of submicroscopic particles in solution (macromolecules, viruses, chromosomes, etc.). A great advantage of this method is its non-destructivity, since neither the preparation nor the agent used for investigation (light) changes the biological structure, which is not the case in electron microscopy and many chemical methods. By means of light scattering further information can be obtained on the mutual interactions of the particles, and the interactions between them and the molecules of the solvent. For solutions containing different kinds of particles, the proportions of the particles of various dimensions and densities can be determined.

The principles of interaction in light scattering can be summarized as follows. The electromagnetic field of the light passing through the solution induces dipole oscillations of the particles. The oscillating dipoles emit light in every direction (cf. section 2.2). In the simplest case, when the interactions between the particles can be neglected, the light waves scattered by the particles in a given direction meet in random phases; consequently, they cannot interfere, and the intensities of the light scattered by the particles are simply added. In every case when the phase difference of the light rays scattered by the individual particles is constant in time, however, they interfere and light diffraction occurs. This latter phenomenon and its practical applications are discussed separately (cf. section 3.5). Electromagnetic waves scattered from various points of the same particle may also produce interference; this internal interference will be dealt with later in this section.

Two types of light scattering are distinguished: Rayleigh and Raman scattering.

1. Rayleigh scattering is also known as elastic or coherent scattering. In this case the wavelengths of the exciting and scattered waves are the same.

(a) For dilute solutions containing (dielectric) particles of much smaller size than the wavelength, the scattered light intensity (I_s) is inversely proportional to the fourth power of the wavelength λ, independently of the direction of scattering:

$$I_s \sim \frac{1}{\lambda^4}$$

In the case of natural illumination, for example, the blue component is scattered 16 times more strongly than the red light with twice the wavelength. This explains the bluish colour of colloid solutions, the blue of the sky, or the red colour of the sunset. Light is scattered by the submicroscopic density changes in the air and by the colloid particles always present in the atmosphere. The intensity of the scattered light also depends on the size (a) of the scattering particles. If $a \ll \lambda$, a simple power relation holds:

$$I_s \sim a^6$$

On the polymerization of macromolecules or the production of aggregates, the light scattering (i.e. the turbidity of the solution) increases rapidly with increasing particle size. In this case the dimensions of the oscillating dipoles can be determined directly from the intensity of the scattered light. This dimension is approximately equal to one of the geometrical dimensions (a) of the particles, e.g. the length of a DNA fibre or the radius of a protein coil.

From the intensity of the scattered light, conclusions can be drawn on the refractive index of the particles, which is closely connected with the particle density. If the dimensions and the density of the particles are known, the molar mass too can be determined.

(b) For particles whose size is comparable with or even larger than wavelength, the dependence of the scattered intensity on the wavelength and the particle dimensions can no longer be described by a simple power equation. The intensity is then highly dependent on the orientation too. However, this spatial distribution permits inferences as to the shape and size of the scattering particle. As an example of this, Fig. 3.17 presents a light scattering curve for a suspension of *E. coli* B bacteria of greater size (about 1×2 μm) than the light wavelength. The orientational inhomogeneity can be seen clearly: measured from the direction of the direct light beam in the angle range 20–140°, the light intensity displays maxima in certain directions ($\approx 40°$, $\approx 60°$ and $\approx 80°$ in the diagram). The inhomogeneity of the distribution (i.e. the positions of the maxima and their intensities) is characteristic of the shape and size of the scattering particle. For comparison, the theoretical scattering of ellipsoidal model particles (shaded "ribbon") is also illustrated. The ribbon represents a set of curves, the individual curves relating to model particles of different sizes: the shorter diameter of the ellipsoids is 0.86–0.92 μm, while the longer one is twice this. A similar comparison of theoretical and experimental curves in other cases too may give acceptable information concerning the geometrical data on the particles.

The intensity distribution of the scattered light depends not only on the geometrical parameters, but also on the distribution of the refractive index and the density within the particles. Thus, the method can give information on the distribution of these quantities as well. For example, the wall thickness of a bacterium cell can be determined in this way.

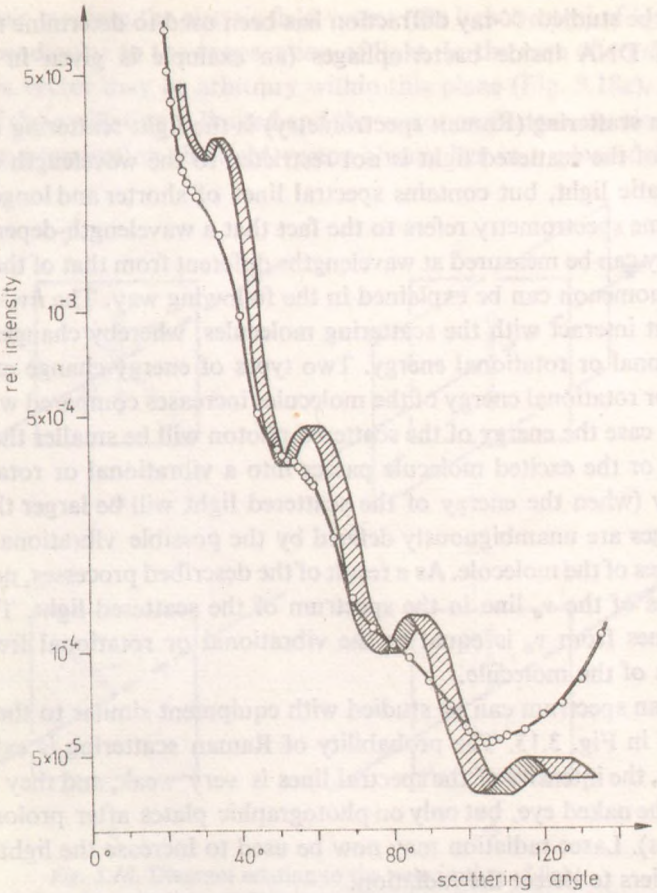


Fig. 3.17. Comparison of experimentally measured light scattering of *E. coli* B bacteria (full curve) with the theoretical scattering of ellipsoid-shaped model particles (shaded ribbon)
The abscissa gives the scattering angle (measured from the direct light beam), and the ordinate the relative intensity of the scattered light (compared to the direct light)

The application of lasers has led to a considerable development in light scattering measurements. The higher intensity of the laser beam improves the sensitivity, and lasers also permit the study of dynamic light scattering. With this technique the hydrodynamic parameters (e.g. the diffusion coefficient) associated with the motion of the particles can be established, and hence the geometrical data and molecular weights can be determined more exactly.

The scattering of other electromagnetic waves can be described similarly as light scattering. From this aspect the ratio a/λ is of interest, since identical values of this are associated with identical scattering laws. With the aid of microwaves for instance, artificial satellites can be investigated, while with X-ray diffraction molecular configu-

rations may be studied. X-ray diffraction has been used to determine the superhelical structure of DNA inside bacteriophages (an example is given in section 3.4.1).

2. Raman scattering (Raman spectrometry) is the light scattering observed if the wavelength of the scattered light is not restricted to the wavelength of the incident monochromatic light, but contains spectral lines of shorter and longer wavelengths too. The name spectrometry refers to the fact that a wavelength-dependent scattered light intensity can be measured at wavelengths different from that of the exciting light.

This phenomenon can be explained in the following way. The $h\nu_0$ photons of the incident light interact with the scattering molecules, whereby changes take place in their vibrational or rotational energy. Two types of energy change exist. Either the vibrational or rotational energy of the molecules increases compared with the original state (in this case the energy of the scattered photon will be smaller than the incident energy $h\nu_0$), or the excited molecule passes into a vibrational or rotational state of lower energy (when the energy of the scattered light will be larger than $h\nu_0$). Both energy changes are unambiguously defined by the possible vibrational or rotational energy changes of the molecule. As a result of the described processes, new lines appear on both sides of the ν_0 line in the spectrum of the scattered light. The distance of these new lines from ν_0 is equal to the vibrational or rotational frequency of the spectral lines of the molecule.

The Raman spectrum can be studied with equipment similar to the luminescence spectrometer in Fig. 3.13. The probability of Raman scattering is extremely small; consequently, the intensity of the spectral lines is very weak, and they cannot be detected with the naked eye, but only on photographic plates after prolonged exposure (several hours). Laser radiation may now be used to increase the light intensity, and photomultipliers to detect the radiation.

The information obtained from Raman spectra does not depend upon the wavelength of the illuminating light. This important circumstance allows the use of Raman spectroscopy in structural research, since the vibrational spectra can be transferred from the experimentally difficult infrared region into the convenient visible or ultraviolet range with a suitable choice of ν_0. A further advantage of this method is that it permits the observation of vibrational and rotational transitions which are forbidden in infrared absorption.

3.3.4. Optical activity

Most biologically important molecules possess characteristic *structural asymmetry*, as they generally have no mirror planes about which the molecular symmetry is invariant *(chiral molecules)*. The polarized absorption spectroscopic methods are especially sensitive techniques for study of these types of structures. However, before a discussion of these methods the basic types of polarized light will be surveyed.

In an isotropic medium the electric field vector (the light vector) of light oscillates in a plane perpendicular to the propagation of light. In the case of *natural light*, the direction of this vector may be arbitrary within this plane (Fig. 3.18*a*). However, if the direction of the oscillation is limited and the vector oscillates only along a straight line, i.e. during propagation the light vector always lies in a plane defined by the

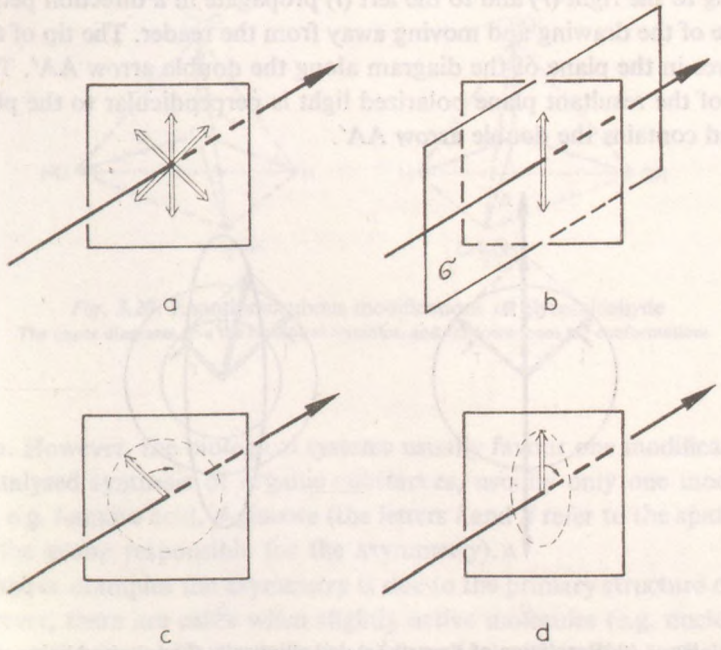


Fig. 3.18. Diagram relating to the polarization of light
a: natural light; *b:* linearly; *c:* circularly; *d:* elliptically polarized light. The solid arrows indicate the direction of the propagation of light; the double arrows indicate the wave vector of the light

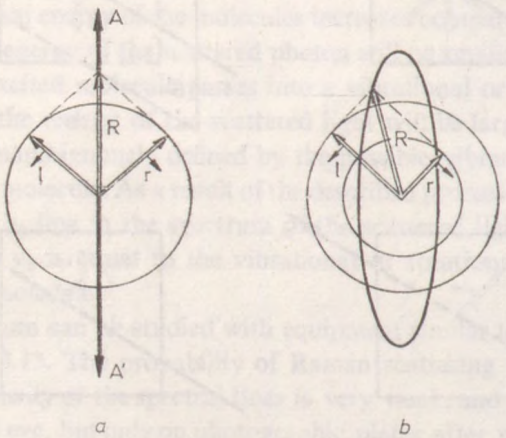
direction of the propagation and the direction of the electric field vector, then the light is *linearly polarized (plane polarized)*. (In Fig. 3.18*b* the plane of oscillation is denoted by σ.[1])

Circularly or elliptically polarized light can also be produced. For circular polarization the magnitude of the light vector is constant, but its direction changes along a circle in a plane perpendicular to the direction of propagation. The velocity of this circulation depends upon the frequency of the light (Fig. 3.18*c*). If the tip of the light vector moves along an elliptical path, the light is elliptically polarized (Fig. 3.18*d*). The circular and the elliptic rotation can be either left or right handed. Both linearly and circularly polarized light may be regarded as special cases of elliptically polar-

[1] For historical reasons the plane perpendicular to this plane is called *the plane of polarization*, i.e. the plane in which the magnetic field vector oscillates normal to the electric field vector.

ized light. In the former case one axis of the ellipse is zero and in the latter the axes are equal.

Plane polarized light may always be regarded as the resultant of two circularly polarized light beams of the same velocity, frequency and amplitude, but rotating in opposite senses. This is demonstrated in Fig. 3.19*a*. The circularly polarized light rays rotating to the right (*r*) and to the left (*l*) propagate in a direction perpendicular to the plane of the drawing and moving away from the reader. The tip of their resultant *R* moves in the plane of the diagram along the double arrow AA′. The oscillation plane of the resultant plane polarized light is perpendicular to the plane of the drawing and contains the double arrow AA′.



a *b*

Fig. 3.19. Resolution of linearly (*a*) and elliptically (*b*) polarized light into circular components with opposite directions

A solution of molecules, asymmetric in the above sense, interacts with the two circularly polarized components of the linearly polarized light in different ways, with the result that the velocities of propagation (refractive indices) will be different. Examination of Fig. 3.19*a* clearly shows that if the two circular components propagate with different velocities, the plane of oscillation of the resultant plane polarized light will be rotated. The extent of rotation is proportional to the difference between the refractive indices of the two components. Substances with chiral structure thus rotate the plane of linearly polarized light passing through them; substances which display this optical property are called *optically active*. If the rotation of the oscillation plane viewed in the opposite direction to that of propagation is clockwise, the rotation is right handed, and in the alternate case it is left handed, denoted by the signs + and —, respectively.

With many substances both left and right rotating modifications can be produced synthetically. The two geometric structures are mirror images of each other (enantiomorphous molecule pairs, Fig. 3.20); they can easily be distinguished by the direction

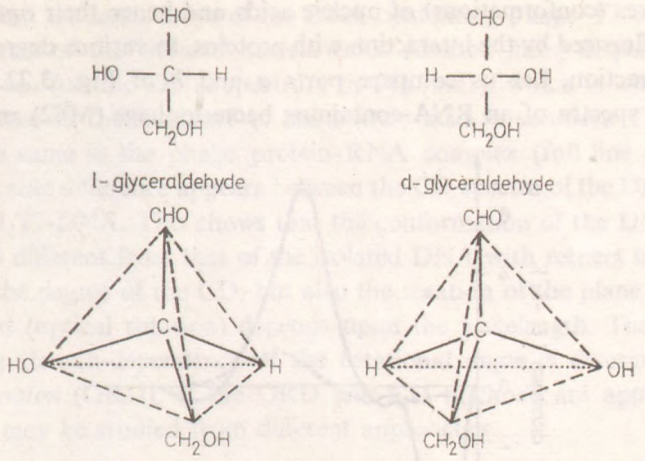


Fig. 3.20. Enantiomorphous modifications of glyceraldehyde
The upper diagrams give the structural formulae, and the lower ones the conformations

of rotation. However, the biological systems usually favour one modification; in the enzyme-catalysed synthesis of organic substances, usually only one modification is produced, e.g. *l*-amino acid, *d*-glucose (the letters *l* and *d* refer to the spatial configuration of the group responsible for the asymmetry).

In the above examples the asymmetry is due to the primary structure of the molecule. However, there are cases when slightly active molecules (e.g. nucleotides) can form macromolecules which have considerable optical activity due to their secondary structure (e.g. DNA, RNA). The helical biological structures (DNA, α-helical proteins) are typically chiral systems, whose optical activity sensitively follows the changes in the secondary structure.

It sometimes occurs that the optically active substance absorbs at the wavelength of the illuminating light. In this case the two circularly polarized components of the plane polarized light not only propagate with different velocities, but are absorbed to different extents. It is easy to see that in this case, when the amplitudes (radii) of the oppositely circularly polarized light components differ, the resultant will be elliptically polarized light (Fig. 3.19*b*). The major semi-axis of the ellipse will be equal to the sum of the radii of the components, and the minor semi-axis to the difference of the radii. This change in the polarization state is called *circular dichroism* (CD), and is measured as the difference of the extinctions of the two circular components. If this difference is plotted against the wavelength, the CD spectrum is obtained. Figure 3.21 shows the CD spectrum of an RNA solution (full line), compared with the CD spectrum of a solution of the nucleotide monomers forming the macromolecule (dashed line). The spectra clearly demonstrate that a considerable part of the optical activity of RNA is due to the secondary structure.

The structures (conformations) of nucleic acids and hence their optical activities may also be influenced by the interaction with proteins, to various degrees depending upon the interaction. Let us compare parts *a* and *b* of Fig. 3.22. The former shows the CD spectra of an RNA-containing bacteriophage (MS2) and its isolated

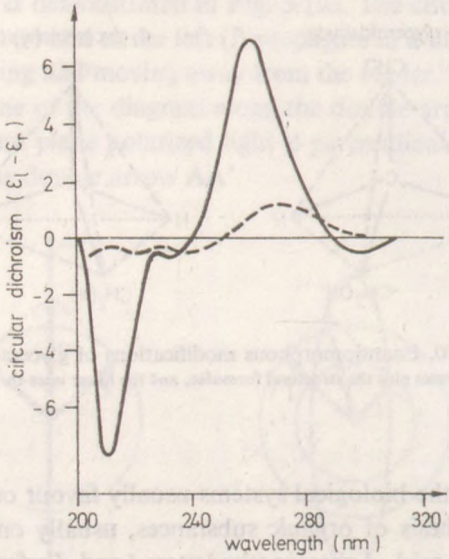


Fig. 3.21. CD spectra

ε_l and ε_r are the molar extinctions of the circular components. The full line relates to the RNA of a plant virus, and the dashed curve to the nucleotide bases forming the RNA (*Samejima* et al., J. Mol. Biol., *34*, 39, 1968; *Cantor* et al., Biopolymers, *9*, 1059, 1970)

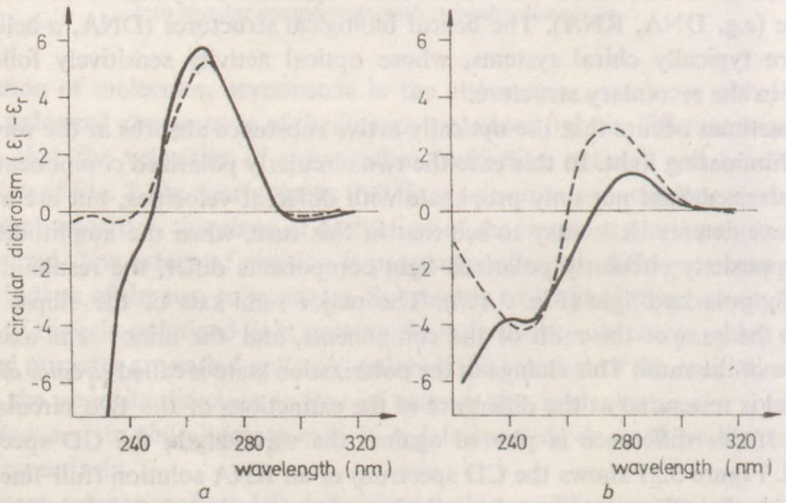


Fig. 3.22. CD spectra of nucleoproteins and isolated nucleic acids (dashed line) in the same solvent

ε_l and ε_r are the molar extinctions of the circular components. *a:* RNA-containing nucleoprotein (phage MS2) and its nucleic acid; *b:* DNA-containing nucleoprotein (phage T7) and its nucleic acid

nucleic acid, and the latter those of the DNA-containing phage T7 and its nucleic acid. The spectra of the isolated nucleic acids (dashed lines) display a maximum at about 270 nm (cf. the DNA spectrum in Fig. 3.21), which is characteristic of the chirality due to the structure of the nucleic acid in solution. This chirality is essentially the same in the phage protein–RNA complex (full line of Fig. 3.22*a*), but a considerable difference appears between the CD spectra of the DNA-containing phage T7 and T7-DNA. This shows that the conformation of the DNA within the phage head is different from that of the isolated DNA with respect to chirality.

Not only the degree of the CD, but also the rotation of the plane of the linearly polarized light (optical rotation) depends upon the wavelength. The technique by which the wavelength-dependence of the rotational angle is determined is *optical rotatory dispersion* (ORD). If the ORD and CD methods are applied, the same phenomenon may be studied from different approaches.

3.4. Diffraction

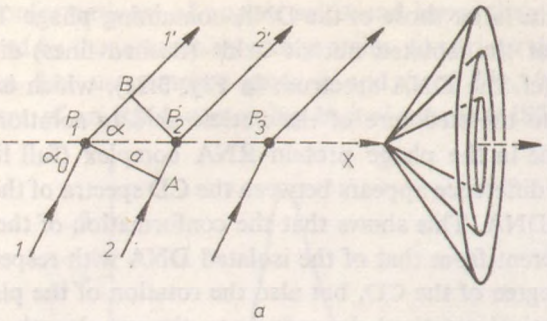
3.4.1. X-ray diffraction

The X-rays incident on atoms are scattered by the atomic electrons. For structure analysis only the coherent scattering is used (cf. section 2.10.2). The diffraction is the stronger the higher the number of electrons associated with the atom, i.e. the larger the atomic number. If the atoms of the irradiated substance are regularly ordered (for instance in crystals) and the atomic distances are of the order of magnitude of the wavelength of the X-radiation, then the rays diffracted by the individual atoms amplify each other in some directions and attenuate each other by interference in other directions. If a single crystal is inserted in the path of the incident radiation and a photographic plate is placed behind the crystal, the parts of the plate where the diffracted rays amplify each other will be darkened (*Laue method;* Fig. 3.23 in the Supplement). The image obtained on a screen is called the *X-ray diffraction pattern.* If the wavelength of the X-radiation is known, the atomic positions and the interatomic distances can be determined from the arrangement interference spots.

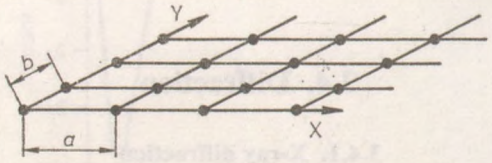
In order to understand the conditions of diffraction, consider Fig. 3.24*a*, which for simplicity depicts a single atomic row (one-dimensional lattice), in which the atoms denoted by the points P_1, P_2, P_3 ... are at the same distance a from each other. The wavelength of the parallel beam is denoted by λ, and the angle of incidence is α_0. Let us consider rays 1 and 2. The angle α denotes the direction in which the X-rays (1′ and 2′) diffracted from the atoms P_1 and P_2 amplify each other by interference. However, amplification is possible only if the difference between paths P_1B and AP_2 is an integral multiple of the wavelength λ, i.e. if

$$a(\cos\alpha - \cos\alpha_0) = e\lambda, \quad e = 0, 1, 2, \ldots, \qquad [3.4a]$$

Condition [3.4a] is obviously satisfied by all diffracted rays which lie on the surface of the cones with half aperture angle α (the *Laue cones*). The axis of the cones is determined by the lattice line X.



a



b

Fig. 3.24. Diagram relating to the production of X-ray diffraction

For a plane lattice another similar condition must be satisfied besides [3.4a] (Fig. 3.24*b*):

$$b(\cos\beta - \cos\beta_0) = f\lambda, \quad f = 0, 1, 2, \ldots, \qquad [3.4b]$$

where b denotes the atomic distance along the Y axis, and β_0 and β are the angles between the Y axis and the directions of the incident and diffracted rays, respectively.

In the case of a space lattice a third equation is also necessary:

$$c(\cos\gamma - \cos\gamma_0) = g\lambda, \quad g = 0, 1, 2, \ldots, \qquad [3.4c]$$

In this equation c is the distance between atomic planes situated above each other along the Z axis, while γ_0 and γ are the angles with Z. Condition [3.4*b*] is associated with Laue cones on the Y axis, and [3.4*c*] with Laue cones on the Z axis.

With space lattices, diffracted beams can be observed only in directions whose angles α, β and γ simultaneously satisfy the three *Laue equations*. Expressed in a different way, the diffracted beams amplify each other only in the direction which is simultaneously the generatrix of the three Laue cones.

Further important information can be obtained from the intensities of the diffraction spots (i.e. from the darkening on the photographic plate), which depends on the amplitude of the interfering waves (scattering amplitude). The amplitude is determined by the interaction of the scattering electron and the X-rays. Thus, by a careful study of the diffraction image the dimensions and shape of the electron shell of the atoms (ions) and the electron density distribution can be determined, thereby providing information on the bonds.

While the *Laue* method can be applied only for single crystals, the method developed by *Debye* and *Scherrer* gives evaluable diffraction patterns on powdered crystals. With the Laue method more or less point-like diffraction spots are observed, whereas the latter method yields *diffraction rings* (Fig. 3.25, Supplement).

The more complex the crystal lattices, the more complex the diffraction patterns. For instance, crystals obtained from protein or DNA molecules display several thousand diffraction spots in the Laue diagrams (Fig. 1.32, Supplement). The determination of such complex crystal structures is possible only with proper ordering and classifying techniques and computerized evaluation. In order to facilitate the evaluation of the diffraction patterns, special techniques are used, in many cases, for instance the method of heavy atom substitution (cf. section 1.5.2).

Structured X-ray patterns may also be obtained from substances exhibiting only short-range order, for instance water, fibrillar biological substances or liquid crystals. However, the greater the disorder, the more blurred the diffraction pattern, the more difficult its evaluation, and the less the information to be obtained from it.

The applicability of the X-ray diffraction method is widely extended by the fact that a diffraction pattern of spatially disordered atoms or molecules (e.g. in a gaseous state) can also be produced, which is the result of interference of radiation scattered on the different parts of the atomic or molecular electron shell. These patterns give information on the electron distribution *within* the atoms or molecules.

In connection with the study of the structure of biological macromolecules and supramolecular systems (e.g. ribosomes), the *small angle diffraction method* should be mentioned. In the case of the substances discussed above for instance, if the distance between the diffracting lattice points is one or two orders of magnitude larger than the wavelength of the diffracted X-rays, the angle of diffraction satisfying the Laue equations deviates from the angle of incidence only slightly. Consequently, the interference patterns obtained from X-rays scattered in a small angle supply information on the arrangement of larger structural subunits (e.g. the ribosome subunits, mRNA), and the effect of smaller structural elements (e.g. water) is not visible in the pattern. This method can be successfully applied to the study of samples containing water.

3.4.2. Electron and neutron diffraction

Electron diffraction. Due to their wave properties, electrons are also diffracted by regularly arranged atoms (ions). The diffraction pattern is similar to that obtained for X-rays and can be evaluated similarly (Fig. 3.26, in the Supplement). The two methods complement each other. Because of their large penetrating power, X-rays are more suitable for the study of thick layers, whereas electrons do not penetrate deeply, and from their diffraction the structure of the surface layers can be determined. As mentioned above, X-rays are diffracted by the electron shells of the atoms, whereas electrons are diffracted mainly by the atomic nuclei. Electrons are especially suitable for the study of surface phenomena (catalysis, adsorption).

Neutron diffraction. By utilization of the wave properties of neutrons (cf. section 1.1), diffraction structural analysis can also be carried out with neutron radiation. For this purpose mainly *thermal neutrons* are used (cf. section 2.14.4), whose wavelength corresponds to the wavelength of the X-radiation used in X-ray diffraction (0.1–0.2 nm). The positions of the interference spots are determined by the distance between the scattering centres (Laue equations), which in this case are the atomic nuclei. While electrons are scattered mainly by heavy nuclei, neutrons are also scattered by protons, and thus neutron diffraction can be used with good results to determine the structures of substances containing hydrogen atoms. Many applications are based on the considerable difference between the scattering amplitudes of hydrogen and deuterium. Thus substitution of hydrogen atoms of special interest by deuterium may lead to the determination of their position. The small angle technique in this case can equally be used for the study of large structural elements (cf. section 3.4.1).

3.5. Other methods

3.5.1. Magnetic resonance spectrometry

This section deals with methods of structural analysis based on the magnetic properties of the atoms.

These methods give information on the static and dynamic conditions in the environment of atoms and atomic nuclei.

It has already been mentioned in section 1.2.2 that a magnetic moment is associated with each non-zero spin momentum and with the non-zero angular momentum of charged particles. Thus, both the nucleus and the electron shell may possess a non-zero resultant magnetic moment. Such atoms undergo ordering in a magnetic field. Only those directions of the magnetic moment are possible for which the projection of the resultant angular momentum along the external magnetic field is $M\hbar$, where M is the *orientation or magnetic quantum number*. The turning of the magnetic moment from one possible direction into another is a process associated with energy change. This means the splitting of the energy levels of the nucleus or the electron shell in the magnetic field: one level is replaced by a number M of *Zeeman levels*. The energy difference between the levels is proportional to the magnetic field strength causing the splitting of the levels. The nucleus or the shell can be excited from a lower to a higher Zeeman level by electromagnetic radiation of frequency ν, which satisfies the relation

$$\Delta E = h\nu \qquad [3.5]$$

where ΔE is the energy difference between the two Zeeman levels.

Resonance can be achieved (i.e. the above equation is satisfied) in two ways. One possibility is to apply a constant magnetic field, in which case ΔE assumes a given value, and the exciting frequency is varied until [3.5] is satisfied. In the second case, irradiation of constant ν is applied and the magnetic field is varied. In practice, mainly the latter is the method of choice. The sample is placed between magnet poles and excited by electromagnetic waves propagating perpendicularly to the magnetic field vector (Fig. 3.27). The radiation coming from the sample is measured with a detector. By gradual increase of the magnetic field strength, [3.5] will be satisfied at a given value, observed via the detector as a minimum in the transmitted radiation, since the sample absorbs the incident radia-

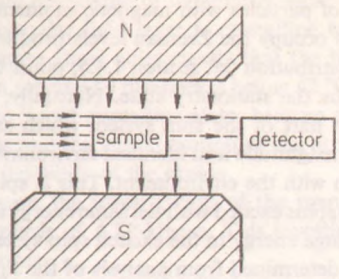


Fig. 3.27. Schematic diagram relating to magnetic resonance spectroscopy
The solid arrows indicate the lines of force of the magnetic field; the dashed arrows refer to the electromagnetic radiation passing through the sample

tion to a higher degree. It is not a simple absorption measurement for besides the transmitted radiaton, that emitted by the sample returning to the ground state is also measured, thus influencing the line-shape of the resulting curve.

Figure 3.28 shows the simple case when M can have only two values; 1/2 and $-1/2$, i.e. only two Zeeman levels are present. Diagram *a* illustrates the above statement that with increasing magnetic field strength the splitting of the energy levels also increases. Diagram *b* depicts the transmittance of the sample as a function of the magnetic field strength, from which the absorption can be determined.

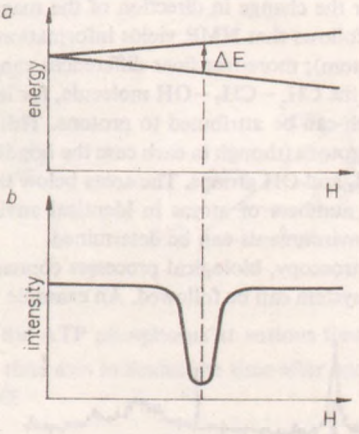


Fig. 3.28. Diagram relating to measurement of magnetic resonance
a: dependence of energy level splitting on magnetic field (H); *b:* dependence of detected intensity on magnetic field (H) for exciting radiation of given frequency ν. The *dashed line* indicates the magnetic field at which the resonance condition $\Delta E = h\nu$ is satisfied

For a deeper understanding of the application of magnetic resonance spectroscopy, one important aspect of this method must be discussed. The return of the system from the excited state to the ground state takes place via *relaxation processes*. Similarly to radioactive decay, a relaxation process follows an exponential law. The time constant of the process is the *relaxation time*, defined as the time in which the deviation from the equilibrium value of the parameter characterizing the state decreases by a factor e. The line-shape of the absorption curve (the height and width of the signal) is determined by the relaxation processes.

In the present case a system of particles with magnetic moments is investigated. Let us regard it as a system of spins. The spins occupy the Zeeman levels produced by the strong magnetic field in accordance with Boltzmann distribution (cf. section 1.4.5, point b) until the exciting electromagnetic radiation no longer perturbs the stationary state. Naturally, electromagnetic radiation of a given frequency excites only that part of the spin system which satisfies the resonance condition [3.5]. These are spins of identical magnitude and identical environment. The excited spins dispose of their excess energy by interaction with the environment. This is spin–lattice relaxation. A different relaxation process can occur if the spins excited with the same energy are situated close to one another. They can then interact and exchange energy in the excited spin system. This is spin–spin relaxation. The two relaxation times can be determined from analysis of the line-shape of the spectrum. These values give information on the dynamic properties (molecular structure, motion) of the investigated system. This method is therefore suitable for study of the conformational changes of macromolecules and macromolecular systems.

Magnetic resonance spectroscopy is a very convenient tool for the investigation of just these dynamic properties. In the following we discuss separately the resonance methods based on the magnetic properties of the nucleus and of the electron shell and mention concrete examples of biological applications.

NMR method. The method based on the magnetic properties of atomic nuclei is known as *nuclear magnetic resonance* (abbreviated to NMR). This method can be used in cases when the resultant nuclear spin, and consequently the resultant magnetic moment, is not zero. This property is characteristic of nuclei whose proton or neutron number, or possibly both are odd.

Some examples of application are listed below.

(a) The energy necessary for the change in direction of the magnetic moment of a nucleus depends upon its environment. It follows that NMR yields information about the nature of the bonding of the studied nucleus (or atom); moreover finer differences can be revealed between bonds of very similar type. In the case of the CH_3-CH_2-OH molecule, for instance, three absorption peaks (resonance peaks) appear which can be attributed to protons. This is a result of the fact that the environments of the individual protons (though in each case the bonding is covalent) differ somewhat from each other in the CH_3, CH_2 and OH groups. The areas below the absorption peaks (resonance peaks) are proportional to the numbers of atoms in identical environments, so that the relative numbers of atoms in different environments can be determined.

(b) By means of NMR spectroscopy, biological processes connected with changes in the structure or dynamics of the studied system can be followed. An example is presented in Fig. 3.29, which

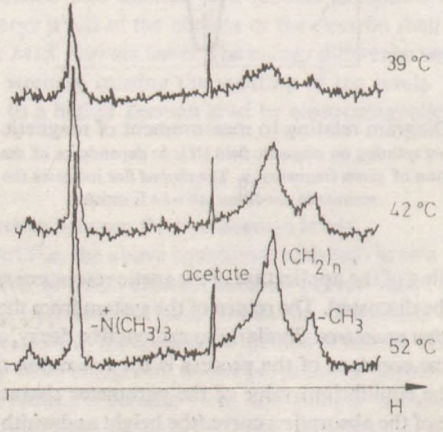


Fig. 3.29. Proton NMR spectrum of a DPL membrane at various temperatures

depicts NMR spectra of a model membrane of dipalmitoyl-lecithin (DPL) molecules, taken at different temperatures. This bimolecular lipid layer is crystalline below 42 °C and liquid-crystalline above this temperature. The curves show quite clearly that the NMR absorption of the hydrogen atoms in the hydrocarbon chain practically disappears below 42 °C. It follows that the hydrocarbon chains, which are flexible in the liquid-crystalline state, become rigid in the crystalline phase. The transmittance of the membranes and the lateral motion of the proteins integrated in the membrane are closely connected with the flexibility of the chains.

(c) The next example concerns the investigation of the manganese ion–ATP complex, which acts as a substrate in the hydrolysis of ATP-kinase. This investigation can be carried out via the NMR spectrum due to phosphorus.

In Fig. 3.30 the return of the spin system of ATP to the stationary state may be followed subsequent to perturbation by a strong magnetic field. The individual peaks are associated with the resonance of the phosphorus atoms in the α, β and γ positions, respectively. In the course of the relaxation process, first the peaks of the phosphorus atoms in the β and γ positions appear, followed by the peak of those in the α position. The shorter relaxation time of the phosphorus atoms in the β and γ positions leads to the conclusion that these atoms are in a more strongly bound environment than the phosphorus atom in the α position. From this it follows that the Mn^{++} ion is coupled to the phosphate groups in the β and γ positions.

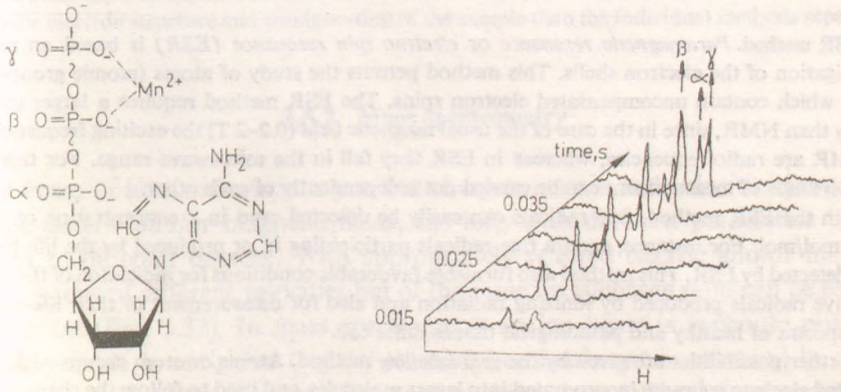


Fig. 3.30. NMR spectrum of the ATP phosphorus at various times during the relaxation process. The time axis indicates the time after excitation

(d) By means of the NMR due to protons, the bound water content of a biological object, e.g. the human eye lens, can be determined.

With gradual cooling of the eye lens, the NMR signal produced by the protons of the water is measured. Since free water freezes at higher temperature than bound water, protons which have lost their mobility as a result of freezing do not give an NMR signal, and the signals observed at lower temperature are therefore due to bound water. The measurements indicate that approximately 25% of the water content of the human eye is present in bound form. (This ratio is lower in some pathological cases.)

(e) Finally, mention may be made of the recently rather frequently used method of incorporating atomic nuclei (atoms) with non-zero magnetic moment into the molecule to be examined. Some biological processes can be studied by this method *(nuclear spin labelling)*.

NMR-tomography (*magnetic resonance imaging; MRI*). The new diagnostic method of NMR imaging has recently been developed. This permits the study of larger samples too, such as the liver, kidney, head or the whole body. With an inhomogeneously varying magnetic field it can be achieved that the resonance condition [3.5] will be fulfilled only in a small volume element of the whole sample, whereas the other parts of the body do not give an NMR signal. On alteration of the inhomogeneous magnetic field, the resonance signal always comes from different sites of the sample, so that the whole body can be scanned.

The signals arising from different sites of a three-dimensional body are analysed with a computer. The computer determines the absorption and the different relaxation times. The intensity is proportional to the proton concentration in the volume-element of the sample from which the signal comes, and the relaxation times are related to its molecular environment. These data are stored in the memory of the computer together with the three coordinates determining the site of origin of the signal. For the physician, sections can be selected and visualized on the display of the computer. These images are similar to those obtained by X-ray imaging techniques, but their interpretation is different, since they reflect different characteristics of the body (proton concentration or molecular environment). Therefore, NMR imaging is more suitable for the examination of soft tissues, while X-ray imaging is used for tissues containing heavy elements too because it is more sensitive for heavier elements. Typical application fields of NMR imaging are the diagnosis of tumours, blood circulation anomalies and the metabolism of soft tissues.

ESR method. *Paramagnetic resonance* or *electron spin resonance (ESR)* is based on magnetic investigation of the electron shells. This method permits the study of atoms (atomic groups, molecules) which contain uncompensated electron spins. The ESR method requires a larger excitation energy than NMR, since in the case of the usual magnetic field (0.2–2 T) the exciting frequencies used in NMR are radiofrequencies, whereas in ESR they fall in the microwave range. For this reason the two kinds of measurement can be carried out independently of each other.

With the ESR method, free radicals can easily be detected even in a concentration of roughly 10^{-11} mol/mol. For instance, certain free radicals participating in or produced by the life processes were detected by ESR. This method also furnishes favourable conditions for indication of the presence of active radicals produced by ionizing radiation and also for measurement of their life-time. The ESR spectra of healthy and pathological tissues differ too.

Further possibilities are given by the *spin labelling* method. Atoms or atom groups with uncompensated electron spins are incorporated into larger molecules and used to follow the changes in their motion and conformation in various biological processes.

With the aid of spin labelling it can be established how and to what degree the motion of a molecule is restricted by its surroundings. The influence of the surrounding lipids on the functions of membrane proteins, for instance, is an essential question, as is the effect of the presence of the proteins on the lipid structures. Among others, nitroxide can be used as a spin label. Figure 3.31*a* depicts the ESR spectrum of an aqueous solution of this compound. If this molecule is bound to the lipid molecules of the membranes for the purpose of spin labelling, its motion is modified by its surroundings. Examinations show that the motion is slower in a protein than in a lipid environment. These results are demonstrated in Fig. 3.31*b–e*. In a pure lipid environment the ESR spectrum is very similar to the spectrum of the freely moving spin labels, i.e. curve *b* is similar to curve *a*. However, an essentially different result is obtained in an environment rich in protein. This can readily be observed by comparing curves *a* and *e*. This type of measurement shows that the membrane proteins are surrounded by a monomolecular lipid envelope, the dynamic properties of which differ from those of the rest of the lipid membrane, since these lipids are almost immobile. Its presence is generally necessary for the activity of the proteins.

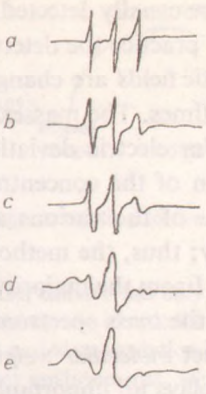


Fig. 3.31. An example of spin labelling. ESR spectra of spin labelling (nitroxide) molecules
a: in aqueous solution; *b:* in a pure phospholipid membrane bound to the lipid; *c–e:* in phospholipid membranes containing proteins in various concentrations (0.49, 0.24, 0.10 mg lipid/mg protein)

ENDOR method. The electron nuclear double resonance (ENDOR) method has been developed from the simultaneous application of ESR and NMR. This method yields more concrete information on the electron structure and configuration of the sample than the individual methods separately.

3.5.2. Mass spectrometry

If a beam of ions with various positive charges, masses and velocities propagates through electric and/or magnetic fields, the ions with different parameters will be deviated to different degrees. With appropriately selected electric and/or magnetic fields, the ions of different velocities but of the same mass/charge ratio can be focused at one point (Fig. 3.32). In mass spectrometry an ion beam is generally produced from the sample investigated (e.g. by electron bombardment in vacuum) in such a way that the bulk of the ions have only one positive charge. Thus, the separation of the ions due to the effect of the electric and/or magnetic fields will characterize their different masses. This separation yields the *mass spectrum*. The ions focused on the

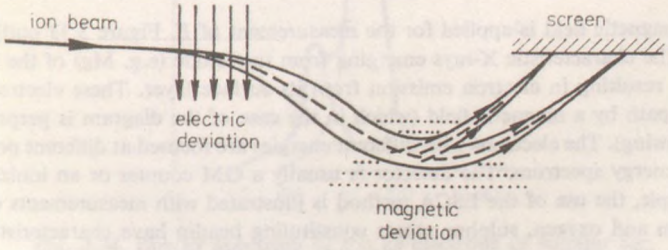


Fig. 3.32. Outline of operation of mass spectrometer
The electric field vectors are parallel to the plane of the drawing, while the magnetic field vectors are perpendicular to it

individual parts of the spectrum are usually detected by means of a secondary electron multiplier provided with a slit. In practice the detector is not moved along the spectrum, but the electric and magnetic fields are changed so that the ions with various masses reach the slit at different times. The masses of the ions are determined from the degrees of the magnetic and/or electric deviation, and the strengths of the detected signals allow determination of the concentrations.

Extremely *small concentrations* of the various atoms and their isotopes can be measured with mass spectrometry; thus, the method permits the detection of trace-elements in biological substances. From the majority of stable small molecules, molecular ions can be produced in the mass spectrometer by electron bombardment, and in this way an extremely exact *molecular weight determination* is possible. The mass spectrometric method also plays an important role as a very sensitive evaluating procedure in the isotope tracer technique (cf. section 2.18). Mass spectrometry is important in *pharmaceutical structure analysis*. In the course of mass spectrometric investigation, not only are molecules ionized by the electron bombardment, but electrically charged fragments are also produced. From these easily identifiable mosaic particles, the macromolecular structure of the original substance can be deduced. One great advantage of the method is that the analysis can be carried out with even a few tenths of a mg of substances.

3.5.3. Electron spectrometry for chemical analysis

Several types of electron spectrometry exist, one is electron spectroscopy for chemical analysis abbreviated to ESCA. In the case of chemical bonds changes taking place in the outer shell electrons may alter the energy states of the electrons in the full core electron shells by 0.1–0.01%. The measurement of these variations thus permits the investigation of the *atomic bonds* and their *changes*. Electrons are ejected from the inner orbitals of the atoms to be investigated by X-ray photons (photo-effect) and their energy is measured with high-precision electron spectrometers. The energy E of the emerging electrons is smaller than the energy $h\nu$ of the X-ray photon. The energy difference (W) between $h\nu$ and E is equal to the bonding energy within the atom, i.e.

$$E = h\nu - W \qquad [3.6]$$

The electron spectrometer measures E, and since $h\nu$ is constant and known, the value of E supplies W directly.

Usually a magnetic field is applied for the measurement of E. Figure 3.33 outlines a magnetic spectrometer. The characteristic X-rays emerging from the anode (e.g. Mg) of the X-ray tube fall on the sample, resulting in electron emission from its surface layer. These electrons are brought into a circular path by a magnetic field (which in the case of the diagram is perpendicular to the plane of the drawing). The electrons with different energies are focused at different points (P, P', ...). producing the energy spectrum. The detector is usually a GM counter or an ionization chamber.

As an example, the use of the ESCA method is illustrated with measurements on insulin. The carbon, nitrogen and oxygen, sulphur atoms constituting insulin have characteristic, well-defined electron spectra. The sulphur atoms can be found in the cystine component of the molecule, two of them being situated between the chains and one in the A-chain. Figure 3.34 depicts the electron spectrum of the $2p$ electrons of the sulphur in normal bovine insulin (diagram *a*), and in the insulin mole-

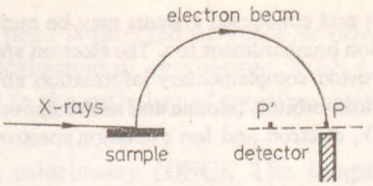


Fig. 3.33. Diagram relating to the ESCA method

cule oxidized with periodate (diagram *b*). Curve *a* has only one peak, which shows that the environments of the sulphur atoms in the molecule are identical insofar as every atom participates in a disulphide bond. Curve *b* shows that the periodate oxidation is selective. The double peak refers to the presence of sulphur atoms in different environments, which means that the treatment does not influence the disulphide bond in the *A*-chain, only the sulphur between the *A* and *B*-chains being oxidized. The ratio of the intensity maxima in the spectrum is 2:1, which corresponds to the ratio of the sulphur atoms between the *A* and *B*-chains of the insulin and the sulphur atom within the *A*-chain.

Besides the energy distribution of the emerging electrons, the angle and time distributions may also yield valuable structural information.

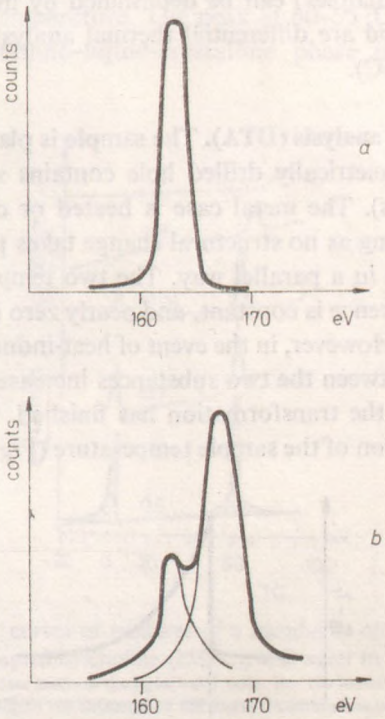


Fig. 3.34. Energy spectrum of the 2*p* electrons of sulphur for (*a*) normal insulin and (*b*) insulin selectively oxidized with periodate
The abscissa gives the bonding energy, and the ordinate the number of measured electrons (*K. Siegbahn* et al., Ann. Phys. *3*, 281, 1968)

The electrons of molecules and condensed systems may be excited not only by X-rays, but by UV radiation and electron or ion bombardment too. The electron spectra obtained by means of the various excitation methods provide complementary information about the structure and allow the location of the individual electron orbitals (atomic and molecular orbitals). In a broader sense electron spectroscopy involves UV, electron and ion excitation spectroscopy too.

3.5.4. Microcalorimetry

The higher order structure of the biologically important molecules (e.g. proteins, nucleic acids, phospholipids) and the systems built up from them (e.g. membranes, chromatin) may be altered considerably by a small change in the external conditions (temperature, pressure, ion concentration); this results in changes in their functions. The high sensitivity is connected with the relatively weak interactions (van der Waals forces, hydrogen bonds) which stabilize the higher order structures. In these cases, and also in more complex systems such as viruses and cells, the structural changes (phase transitions) resulting from small temperature changes can be sensitively studied by microcalorimetric methods. The transition temperatures and heats (more exactly the transition enthalpies) can be determined by microcalorimetry. The two main types of this method are differential thermal analysis (DTA) and differential scanning calorimetry (DSC).

1. Differential thermal analysis (DTA). The sample is placed into a hole in a metal case, while another symmetrically drilled hole contains some reference substance (for instance, glass beads). The metal case is heated or cooled at a constant rate (0.1–50 °C/minute). As long as no structural change takes place, the temperatures of the two materials change in a parallel way. The two temperatures may differ from each other, but their difference is constant, and nearly zero if the heating (or cooling) rate is sufficiently small. However, in the event of heat-induced structural changes the temperature difference between the two substances increases, and it decreases to the original value only after the transformation has finished. If the temperature difference is plotted as a function of the sample temperature (Fig. 3.35), a peak is obtained

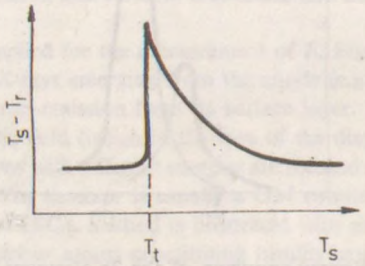


Fig. 3.35. DTA curve of an endothermic phase transformation
T_s is the temperature of the sample, T_r the temperature of the reference substance, and T_t the transition temperature

at the transformation; this peak is directed upwards or downwards, depending upon whether the reaction is endothermic or exothermic. With a uniform heat supply, the peak height is proportional to the enthalpy of transformation.

2. Differential scanning calorimetry (DSC). The temperatures of the reference substance and the sample are kept at a constant value in this method by appropriate selection of the heating rate. In the case of DSC curves the abscissa depicts the specimen temperature, and the ordinate the difference between the heating rates for the reference substance and the sample necessary for the constant temperature. The heating rate is the heat introduced into the system per unit time. By means of DSC the temperature-dependence of the heat capacity, and the order, rate constant and activation energy of the reaction can be determined.

One application of this method, the determination of the bound water content of biological systems by DSC, is mentioned below. The left side of Fig. 3.36 relates to this method. The peak at 0 °C is connected with the free water-ice transformation. The peak height increases in proportion to the water content. With an 80% lipid content this peak disappears, which means that the system contains only bound water, which freezes at a lower temperature. The peak at 60–65 °C on the right side of the figure relates to the crystalline–liquid-crystalline phase transition of the DSPC membrane.

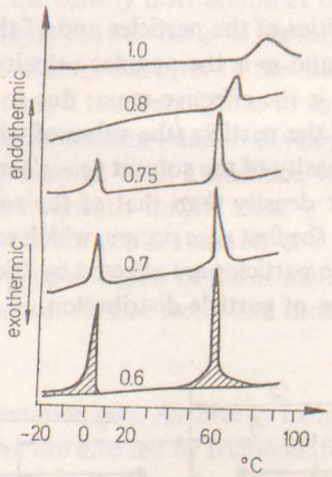


Fig. 3.36. DSC curves of mixtures of a membrane constituent lipid, distearoylphosphatidylcholine (DSPC), and water in various ratios
The numerical data indicate the lipid/water ratio. For the lowest curve the area corresponding to the enthalpy of the phase transition has been shaded

3.5.5. Sedimentation

The measurement of the sedimentation rate of suspended particles, and after the termination of this process, the investigation of the particle distribution in the solution allows conclusions about the dimensions, shape, density, molecular weight, etc. of the particles. Further, it is possible to separate particles with various parameters. The sedimentation method is used in biology to study cells, cell-components, viruses, molecules, etc. In most cases the gravitational field strength is not sufficient. Instead, the centrifugal forces of rotating systems are used; in suitable equipment (*centrifuges*) these exceed the gravitational force by several orders of magnitude. For instance, in a centrifuge (ultracentrifuge) performing approximately 1000 revolutions per second, the centrifugal force may be 10^5 times larger than the gravitational force.

1. Investigation of particle distribution. At the beginning of the centrifugation the particles migrate in the solution in accordance with the generalized Archimedes law, in the direction which is the resultant (F) of the centrifugal force (F_{cf}) and the lifting force (F_l) acting in the opposite direction to F_{cf}.[2] The magnitude of the resultant force is given by the relation

$$F = (\varrho - \varrho')V r\omega^2 \quad \text{or} \quad F = mr\omega^2 \qquad [3.7]$$

where ϱ and ϱ' are the densities of the particles and of the solvent, respectively, V is the volume of the particle, and ω is the angular velocity of the rotation (right side of Fig. 3.37). $m=(\varrho-\varrho')V$ is the effective mass; due to the lifting force, this is not equal to the actual mass of the particle (the value of which is ϱV). Particles whose density is higher than the density of the solvent ($\varrho > \varrho'$) migrate towards the bottom, while particles with a lower density than that of the solvent move in the opposite direction. In practice mainly the first case occurs, which explains the name *sedimentation*. Besides these forces, the particles are affected by frictional forces, but these can be neglected in investigations of particle distribution.

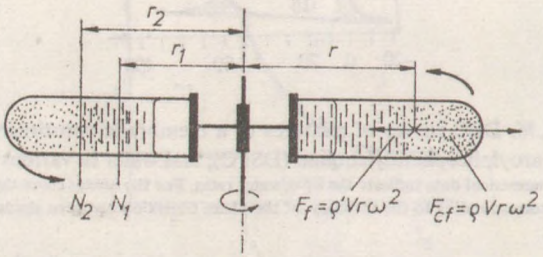


Fig. 3.37. Outline of centrifugation

[2] The lifting force is the compressive force of the solvent on the particles.

If the centrifugation is continued for a sufficiently long period, the migration (sedimentation) of the particles ceases, though because of the thermal motion they do not all cluster tightly at the bottom of the centrifuge tube. Their distribution can be described by the *Boltzmann relation,* which also describes the distribution of dust and other particles suspended in air as a function of altitude. In the present case the potential energy of the particles in the rotating system has to be considered. According to Boltzmann:

$$\frac{N_1}{N_2} = \exp - \left[\frac{\frac{1}{2} m\omega^2 (r_2^2 - r_1^2)}{kT} \right] \qquad [3.8]$$

where k is the Boltzmann constant, T is the absolute temperature, and N_1 and N_2 are the equilibrium concentrations of the particles at distances r_1 and r_2, respectively, from the axis of rotation (left side of Fig. 3.37). The numerator of the exponent contains the difference between the potential energies of the particles of effective mass m due to the change of their distance. It follows from [3.8] that larger particles (for instance cells, or larger cell components) collect at the bottom of the centrifuge tube even at not too high rotation rates. With smaller particles (e.g. macromolecules), even if an ultracentrifuge is used, the particle distribution according to [3.8] must be taken into consideration.

It has been assumed that the density distribution of the suspending medium does not change during the centrifugation, i.e. that ϱ' is the same at every site in the centrifuge tube. In practice this is usually the case. In the interest of separating particles with different densities, however, it is advantageous to use a medium in which a density gradient described by the Boltzmann relation develops due to the centrifugation *(centrifugation in a density gradient)*. Such a medium, for instance, is a solution of some heavy metal salt (most frequently CsCl). Thus, ϱ' in [3.7] is not constant, but increases with the distance from the axis of rotation. It is clear from [3.7] that the particles of density ϱ collect in a given region of the centrifuge tube, where $\varrho = \varrho'$. The particles of higher density collect further from, and those of lower density closer to the axis of rotation.

2. Measurement of sedimentation rate. As already mentioned, in the sedimentation process the migrating particles are affected by frictional forces besides the centrifugal and lifting forces. If the resultant of these three forces is nearly zero, the constant rate of sedimentation (v) may be regarded as proportional to the centrifugal acceleration ($r\omega^2$), i.e.

$$v = sr\omega^2 \qquad [3.9]$$

where the proportionality factor s (the sedimentation constant) has dimensions of time. [3.9] is usually satisfied in practice. Some centrifuge types permit the measurement of v and, if r and ω are known, s can be determined. From the value of the sedi-

Table 3.1

Some data on macromolecules and viruses

Particle	Molecular weight	$S_{20,w}$ (svedberg unit)
Myoglobin	1.7×10^4	2.0
Haemoglobin	6.8×10^4	4.0
Botulinus toxin	9.5×10^5	17.0
E. coli ribosome	2.8×10^6	69.1
Poliomyelitis virus	6.7×10^6	120.0
Phage T2	2.0×10^8	900.0

mentation constant, conclusions can be drawn as to the dimensions of the particles (or of their hydrate sheath), their shape and their molecular weight. Since the viscosity of the solvent also influences the sedimentation constant, s depends very sensitively upon temperature. For this reason the value of s is usually given related to water at 20 °C. In this case, instead of s the proportionality factor is denoted by $S_{20,w}$. Its unit is the *svedberg* which corresponds to 10^{-13} s. For informative purposes, Table 3.1 lists sedimentation constants and molecular weights for some biological macromolecules and viruses.

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4. TRANSPORT PROCESSES. THERMODYNAMIC BASIS OF LIFE PROCESSES

This chapter is divided into three main parts. We first treat the transport processes which are of outstanding biological importance, while in the second part the basis of thermodynamics is dealt with. Finally, the material in the first two parts is used to discuss the biophysical aspects of membrane transport phenomena.

4.1. Flow of fluids and gases

In the life processes, especially in those of more highly developed organisms, the circulation of various fluids and gases is of prominent importance. Consider for instance blood circulation or respiration. These processes can be modelled from a physical viewpoint by the flow of fluids and gases in tubes.

4.1.1. Basic concepts

As long as the flow velocity is below a value of approximately 50 m/s, the compressibility of gases plays practically no role in flow phenomena. For this reason, both gases and fluids are regarded as incompressible. Since the velocities do not exceed the above critical value, the flow of gases can be discussed together with the flow of fluids. Though only fluids are mentioned in the following treatment, the results also hold for gases.

The discussion is restricted to flow in rigid-walled tubes; tubes with elastic walls will be dealt with in section 4.1.6. The flow of fluids is characterized by the fluid volume flowing through the tube cross-section in unit time, or more exactly by the *volume current strength* (I; I can also be defined as the intensity of the current). By definition, we have

$$I = \frac{\Delta V}{\Delta t} \qquad [4.1a]$$

where ΔV denotes the volume of fluid flowing through the tube cross-section in time Δt.

With *ideal* fluids, i.e. frictionless (and incompressible) fluids, the flow velocity is the same at every point of the tube, and a simple equation describes the relation between this common velocity $\bar{v}$ and the current intensity I. If a fluid volume element $\Delta V = q\Delta s$ flows through a tube of length Δs and cross-section q during time Δt, from the definition of current intensity:

$$I = q\frac{\Delta s}{\Delta t} = q\bar{v} \qquad [4.1b]$$

In real fluids the velocity differs at the individual points of a given cross-section of the tube, being maximum in the tube axis and decreasing from the axis towards the tube wall. In these cases it is customary to work with the *mean velocity* belonging to the given cross-section, as defined by [4.1b]. Consequently, [4.1b] is considered as valid for real fluids too, and the *mean velocity* is given by

$$\bar{v} = \frac{I}{q} \qquad [4.1c]$$

i.e. the mean velocity is the quotient of the current intensity and the cross-section.

If the characteristic quantities of the current (velocity, current intensity, pressure) are time-independent, and at most vary from place to place, the current is said to be *stationary*. It is clear that for stationary currents the velocity is larger with a small than with a large tube cross-section: the velocity is inversely proportional to the cross-section.

4.1.2. Bernoulli's law

In this section we study the pressure distribution of ideal fluids undergoing stationary flow through tubes of different cross-sections (Fig. 4.1). For simplicity we consider a horizontal tube. The pressure (or more exactly the hydrostatic pressure) is given by the height of the fluid column in a vertical side-tube. According to Bernoulli's law, at any point in the tube

$$p + \frac{1}{2}\varrho v^2 = \text{constant} \qquad [4.2a]$$

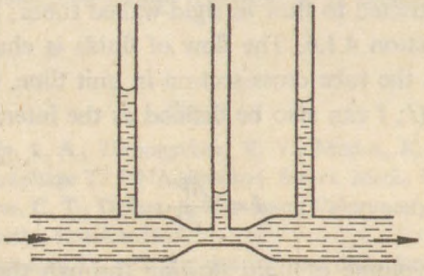

Fig. 4.1. Diagram relating to Bernoulli's law

where ϱ denotes the fluid density, while p and v are its pressure and velocity, respectively. p in [4.2a] is often referred to as the static pressure, and the quantity $\frac{1}{2}\varrho v^2$ as the *dynamic pressure;* the sum of these two quantities is the total pressure. Thus, from Bernoulli's law the *sum of the static and the dynamic pressures is constant and equal to the total pressure.*

If the tube is not horizontal, the change in the potential energy of the fluid particles due to gravitation must also be considered and instead of [4.2a] we have

$$p+\frac{1}{2}\varrho v^2+\varrho g h = \text{constant} \qquad [4.2b]$$

Bernoulli's law essentially expresses the *law of conservation of energy* for fluids.

4.1.3. Internal friction. Stokes' law

Here again fluids will be discussed, though the results are also valid for gases. If a body moves in a liquid medium, or the medium flows with respect to the body, a force due to friction is exerted on the body. The friction between the medium and the surface of the body is referred to as *external friction,* while the displacements of the layers of the medium with respect to each other give rise to *internal friction.* In practice the fluid usually wets the surface of the body, which means that a liquid layer of given thickness (sometimes only a monomolecular layer) adhering to the surface moves together with the body. In the relative motion of the body and its liquid environment, fluid generally moves on fluid and only internal friction occurs.

The internal friction is characterized by the *internal friction coefficient,* i.e. the *viscosity.* The introduction of this concept is associated with a phenomenon important in practice. The viscosity can be studied by letting spherical bodies fall in some liquid. As the body falls, it is first accelerated by gravitation, but later the acceleration gradually decreases to zero and the body attains a constant velocity. This phenomenon is explained in that the frictional force acting on the body increases with its increasing velocity. Finally, when the frictional and driving forces become equal, the velocity of the falling body becomes constant. Experimental evidence demonstrates that in most cases the internal frictional force (F_f) is proportional to the relative velocity (v) of the body:

$$F_f \sim v \qquad [4.3a]$$

The frictional force depends upon the shape of the body and the nature of the medium. In the case of spheres, the form factor is the radius of the sphere (r), and the internal friction is proportional to r. The nature of the medium is included in the proportionality factor; for spheres (without going into detail) this can be expressed by the quantity $6\pi\eta$. Consequently,

$$F_f = 6\pi\eta r v \qquad [4.3b]$$

The factor η is the *internal friction coefficient* or simply the *viscosity*, its unit is Pa s. For instance, the viscosity of water at 18 °C is 1.1 mPa s, and that of the air at atmospheric pressure is 0.018 mPa s. The viscosity of an "easily flowing" fluid is small, whereas viscous fluids have high viscosities.

As mentioned above, the liquid layer adhering to the surface moves together with the body, though its effect decreases with increasing distance from the body. The fluid layer in which this effect is still observable is called the *boundary layer*. For water the boundary layer has a thickness of a few mm, whereas for fluids of higher viscosity the boundary layer is thicker, e.g. for blood a few cm.

The reciprocal of the viscosity is the fluidity. The quantity η is frequently referred to as *dynamic* viscosity and the quotient η/ϱ as *kinetic* viscosity (ϱ denotes the density of the liquid).

The viscosity depends very sensitively upon the temperature. For gases, the viscosity grows proportionally to the square root of the absolute temperature T. This can be qualitatively explained in that the interaction of the gas layers sliding on each other is the more intensive, the higher the mean thermal velocity of the molecules. For fluids, however, the viscosity decreases with increasing temperature. The mechanism of internal friction is different for liquids since the molecular gaps, vacancies, play an essential role in the displacement of the liquid layers on one another. With more vacancies the molecules can jump more easily into the adjacent vacancies, with the result that with an increasing vacancy concentration the mutual displacement of the layers becomes easier and the viscosity decreases. This also means that the viscosity is nearly inversely proportional to the corresponding Boltzmann factor, i.e. more exactly

$$\frac{1}{\eta} \sim T e^{-\frac{\varepsilon}{kT}} \qquad [4.3c]$$

The letter ε here denotes the *activation energy of molecular migration*, whose value in the case of liquids is a few tenths, or frequently only a few hundredths of an eV. (The factor T before the exponential term generally plays only a minor role as compared to that of the rapid exponential change.)

[4.3b] has been used to introduce the concept of viscosity, but it is also frequently used as a special relation called Stokes' law. By its aid the constant velocity v attained by a sphere of radius r and density ϱ' falling in air of viscosity η and density ϱ can be calculated, this will be

$$v = \frac{2g}{9\eta}(\varrho' - \varrho)r^2 \qquad [4.3d]$$

This relation explains the relatively low falling velocity of small fog droplets or dust particles in the air. The equation can also be used for viscosity measurements or to determine the radius of spherical particles (such as, for instance, colloidal particles or macromolecules).

Finally, a further remark is made in connection with [4.3a]. In the case of motion at constant velocity, the driving and the frictional forces differ from each other only in sign, and consequently the driving force F_d is proportional to the velocity. This relation can be written in the form

$$v = uF_d \qquad [4.4a]$$

where the coefficient u is called the *mobility*. The value of u gives the mean velocity of a colloid particle or macromolecule moved in some medium by unit driving force. In the case of spherical particles we have

$$u = \frac{1}{6\pi\eta r} \qquad [4.4b]$$

4.1.4. The Hagen–Poiseuille law

Consider a fluid flowing in a horizontal tube of constant circular cross-section (Fig. 4.2). Let the volume of fluid flowing across the cross-section $r^2\pi$ in time Δt be ΔV. The pressure on the tube wall (static pressure) is measured by the height of the fluid column in a vertical tube connected to the horizontal system. Let the pressures at the two ends of the horizontal tube length l be p_1 and p_2, respectively. The pressure decrease per unit length, as expressed by the quantity $(p_1-p_2)/l$, is called the *pressure*

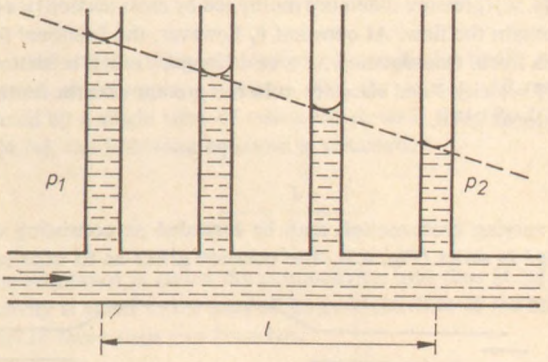

Fig. 4.2. Diagram relating to the Hagen–Poiseuille law

drop. According to the Hagen–Poiseuille law, ΔV is proportional to the flow time, the pressure drop and the fourth power of the tube radius, and inversely proportional to the viscosity (η) of the fluid. Without going into a detailed discussion, the proportionality factor is $\pi/8$. Consequently

$$V = \frac{\pi}{8}\frac{r^4}{\eta}\frac{p_1-p_2}{l}\Delta t$$

and [4.5a]

$$I \equiv \frac{\Delta V}{\Delta t} = \frac{\pi}{8}\frac{r^4}{\eta}\frac{p_1-p_2}{l}$$

The pressure distribution along the tube length is characterized by the quantity dp/dl, called the pressure gradient. For identical cross-sections the pressure gradient is constant in the various regions, and for this reason the pressure gradient can also be written in the form $(p_2-p_1)/l$. In [4.5a] we have $-(p_2-p_1)/l$, since $p_1>p_2$. This means that the intensity of the volume flow is proportional to the negative pressure gradient (cf. section 4.6.1).

To measure the tube resistance (frictional resistance), various quantities are used:

(a) [4.5a] can be written in the following form

$$p_1-p_2 = RI \qquad [4.5b]$$

where

$$R = 8\pi\eta\frac{1}{\pi^2 r^4} \qquad [4.5c]$$

and the resistance is characterized by the quantity R. [4.5b] is similar to Ohm's law for electric current, which describes the relation between the potential difference, the electric current intensity and the resistance of the conductor. The Hagen–Poiseuille law expresses a similar relation between the pressure difference, the liquid flow intensity and the frictional resistance.

The electric resistance is proportional to the length of the conductor, and inversely proportional to the tube cross-section. The frictional resistance R is proportional to the length of the tube and inversely proportional to the square of the tube cross-section.

(b) In the case of a circular cross-section, from [4.1c] $I=\bar{v}r^2\pi$, and thus [4.5a] can be rewritten in the form

$$(p_1-p_2)r^2\pi = 8\pi\eta l\bar{v} \qquad [4.5d]$$

The left-hand side of [4.5d] (pressure difference multiplied by cross-section) is equal to the compressive force required to maintain the flow. At constant $\bar{v}$, however, the frictional force acting against the current is equal to this force. Consequently, a tube of length l exerts a frictional force $8\pi\eta l\bar{v}$ against the flow of a liquid of velocity $\bar{v}$ and viscosity η. In the present case the frictional resistance is characterized by this frictional force.

Further remarks

(a) A tube with varying cross-section may be regarded as consisting of parts with different cross-section connected in series (Fig. 4.3). The question arises as to what law governs the flow in

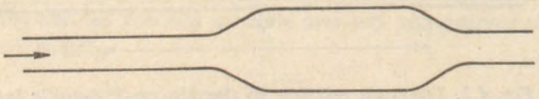

Fig. 4.3. Resistances connected in series

this type of tube system. The cross-sections of the individual tube segments are constant, which means that for each tube segment of the system [4.5a] and [4.5b] can be applied. Without going into details, calculations relating to the overall tube system yield the equation

$$p_i+\frac{1}{2}\varrho v_i^2-\left(p_0+\frac{1}{2}\varrho v_0^2\right) = (R_1+R_2+\ldots+R_n)I \qquad [4.6]$$

where I is the constant current intensity, R_1, R_2 ... R_n are the resistances calculated from [4.5b], and p_i and v_i and p_0 and v_0 are the static pressure and the velocity at the points of inflow and outflow, respectively. The left-hand side of [4.6] gives *the total pressure difference* between the ends of the tube system. The sum of the partial resistances is called the total resistance, and thus [4.6] expresses the fact that the total pressure difference between the tube ends is equal to the product of the current intensity and the total resistance. [4.6] may be regarded as the generalized Hagen–Poiseuille law, which includes [4.5b] as a special case. [4.6] reduces to [4.5b] if the dynamic pressures at the ends of the tube are negligible as compared to the static pressures. Similarly, [4.5b] results if the tube cross-sections are the same, at least at the ends, i.e. when $v_i=v_0$.

(b) In Fig. 4.4 the sum of the current intensities in the branches is equal to the current intensity in the main branch, i.e.

$$I = I_1+I_2 \qquad [4.7a]$$

From [4.5b] the pressure difference between the points A and B is equal to the product of the current intensity and the resistance, i.e. to I_1R_1 and I_2R_2, respectively; consequently

$$I_1R_1 = I_2R_2 \quad \text{and} \quad \frac{I_1}{I_2} = \frac{R_2}{R_1} \qquad [4.7b]$$

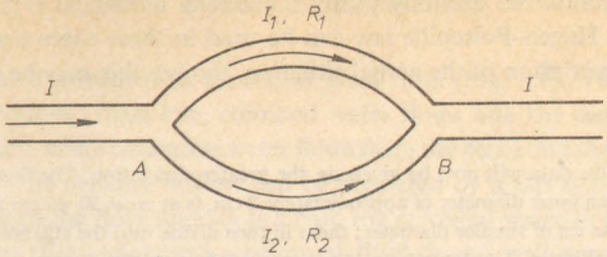

Fig. 4.4. Resistances connected in parallel

This means that the current intensities are inversely proportional to the resistances. The current intensity I and the pressure difference associated with the section AB remain unchanged if the branches are substituted by a single tube of resistance R, such that $IR = I_1R_1$ and $IR = I_2R_2$. From these relations and [4.7a], the following equation is obtained:

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} \qquad [4.7c]$$

The reciprocal of the resistance is called the conductivity, and thus [4.7c] expresses the fact that the resultant conductivity is equal to the sum of the conductivities of the individual branches. This also holds for systems of more than two branches.

So far, the viscosity has been regarded as having a constant value characteristic of the fluid and changing only with the temperature. However, there are some fluids whose viscosity also depends upon the pressure inducing the flow. The former group of fluids are called the *normal* or *Newtonian fluids,* while the latter anomalous or *non-Newtonian.* Pure liquids and true solutions belong in the first group, whereas the second group includes colloid solutions, emulsions and suspensions. In the latter case the dispersed particles are platelets or fibrillar, and only a loose connection exists between them and the dispersing medium. The pressure change inducing the flow orders the elongated particles and disrupts the loose structure between them with the consequence that in both cases the viscosity of this type of fluid decreases with increasing pressure drop. Blood behaves anomalously; at body temperature its viscosity is roughly 4.5 mPa s in the greater arteries and 2 mPa s in the smaller arteries.

The Hagen–Poiseuille law may have various applications, depending upon the known quantities, which in turn allow the unknown ones to be found. Depending upon the nature of the problem, this law may be used, for example, to determine the viscosity, pressure distribution, and so on. It must be stressed, however, that the Hagen–Poiseuille law holds for the stationary flow of Newtonian fluids only if their velocity is below a certain critical value (cf. section 4.1.5). A small cross-section means that the tube radius is smaller than the boundary layer; in the case of water, for instance, this critical value is at most a few mm, and for blood at most 1–2 cm.

The above conditions are only partly satisfied in biological experiments and for this reason the Hagen–Poiseuille law can be used in these cases only to provide an approximate description of the actual situation, though this may be useful as a starting point.

Some informative data will now be given on the greater circulation. The flow velocity of blood in the aorta, with an inner diameter of approximately 2 cm, is at most 30–40 cm/s. The great arteries branch off into arteries of smaller diameter; these in turn divide into the still smaller arterioles, and finally into the capillaries. The branching can be regarded as a tube system connected in parallel, whose total cross-section increases with increasing branching. The flow velocity in the capillaries is only about 0.05–0.08 cm/s, from which it follows that the total cross-section of the capillaries is approximately 600–800 times larger than the cross-section of the great arteries. The capillaries unite into venules, and these into veins, which results in the decrease of the total cross-section and consequently in an increase of the flow velocity. For example, the flow velocity in the vein joining the right atrium is 6–14 cm/s. On contraction, the pressure in the left ventricle increases to be about 13–16 kPa above atmospheric pressure, whereas in the vein flowing into the right atrium the pressure is atmospheric. Figure 4.5 depicts the distribution of the pressure above 101 kPa along the greater circulation. The pressure is seen to decrease considerably in the arterioles and the capillaries, due to the large frictional resistance of these systems. At first sight it might appear surprising that the frictional resistance is large in the arterioles and the capillaries, whose total cross-section is high. In order to explain this fact, the capillary system may be modelled by n tubes of identical radius r,

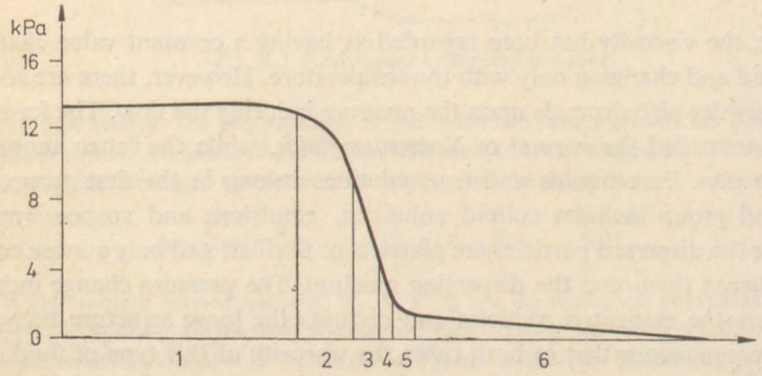

Fig. 4.5. Pressure drop in the greater circulation
1: great arteries; *2:* small arteries; *3:* arterioles; *4:* capillaries; *5:* venules; *6:* veins. The ordinate gives pressure values above 101 kPa in kPa units

connected in parallel. From [4.5c], the resistance of a single tube is inversely proportional to r^4. It follows from [4.7c] that the resultant resistance of n tubes connected in parallel is inversely proportional to nr^4. Since the total cross-section q is proportional to nr^2, the resultant resistance of the tube system is inversely proportional to qr^2. Thus, it is quite conceivable that, even with a large q, qr^2 will be small if r is sufficiently small, and consequently the resultant resistance will also be small. This is the situation with the arterioles and capillaries.

4.1.5. Laminar and turbulent flow

Let water flow downwards in a vertical glass tube (Fig. 4.6). The water source consists of two vessels; from one, coloured water flows into the centre of the tube through an opening, while colourless water flows from the other into the part surrounding the opening. The velocity of flow can be regulated by a tap at the lower end of the tube. As long as the flow velocity is small, the coloured water does not mix with the colourless one (Fig. 4.6*a*), and a coloured fluid-thread well separated from its surroundings can be observed in the tube axis. This type of flow is called *layered* or *laminar* flow. If a given velocity is exceeded, however, rotations (eddies) are added to the unidirectional motion of the fluid particles and a confused flow results (Fig. 4.6*b*). The flow pattern changes continually. This type of flow is called *turbulent*

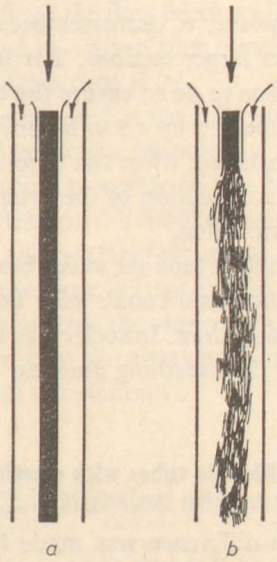

Fig. 4.6. a: Laminar flow; *b:* turbulent flow

flow. Everything discussed in the previous sections refers only to laminar flow. Turbulence increases the frictional resistance, and the statement that the internal frictional force is proportional to the velocity as expressed in [4.3] and [4.4] and in the Hagen–Poiseuille law [4.5d] not longer holds. In the case of turbulence the frictional force is approximately proportional to the square of the flow velocity; the compressive forces maintaining the flow perform work against the frictional forces. Since the frictional resistance increases with the occurrence of turbulence, more work is required to maintain the same current intensity.

The velocity above which laminar flow passes over into turbulent flow is the *critical velocity*. From the investigations of Reynolds, the critical velocity v_c depends

upon the viscosity η and the density ϱ of the fluid, and upon the radius r of the tube:

$$v_c = Re \frac{\eta}{\varrho r} \qquad [4.8]$$

The dimensionless factor *Re* is called the *Reynolds number*, whose value is 1160 for smooth-walled tubes: *Re* is smaller for tubes with rough walls. Thus from [4.8] the flow of water (at 18 °C) in a glass tube 1 mm in radius becomes turbulent only above a velocity of 127 cm/s; in the case of a tube with a radius of 1.0 cm, the critical velocity is one-tenth of this value: $v_c = 12.7$ cm/s. The critical velocity of blood in a smooth-walled tube 1 cm in radius would be 50 cm/s; the actual value in the blood vessels is generally smaller than this.

Under healthy conditions, the vascular flow of the blood is laminar. Turbulence occurs only at some places, e.g. in the aorta behind the semilunar cardiac valves. In certain pathological cases, however, v_c decreases because of the decrease in η, and the turbulence may extend over larger sections. The larger the sections of turbulent flow, the more work the heart has to do to ensure the blood supply.

Turbulent motion is accompanied by a low humming sound, which can be heard in the artery of the arm, for instance, when the blood pressure is taken. This sound is due to the fact that as the cross-section of the artery decreases the flow velocity increases and exceeds the critical value.

The air flow in the nasal canals is laminar under healthy conditions. Under pathological conditions, however, the nasal canals may become so narrow that the air flow becomes turbulent in some sections. In such cases the breathing becomes difficult, resulting in increased work by the breathing muscles.

4.1.6. Flow in tubes with elastic walls

In the previous section, no difference was made between the flow processes in tubes with rigid or with elastic walls, for under conditions of stationary flow the discussed relations can be applied to both cases. Though an elastic tube yielding to pressure expands at the onset of flow, the geometrical dimensions (radius, length, etc.) do not change in the course of flow after it has become stationary. Of course, the new geometrical values of the tube formed during stationary flow must be inserted into the relations describing the flow.

The situation is considerably more complicated in the case of fluctuating pressure values. Instead of a quantitative discussion, we shall be satisfied with a description of an experiment which reveals the essential differences between tubes with rigid or with elastic walls. In Fig. 4.7 the glass tube protruding from a water vessel branches into two parts. One branch is connected to a rubber tube *A*, and the other via a short rubber connection to a glass tube *B*. The cross-sections are selected so that stationary flow of identical intensity is produced in both tubes. If the rubber

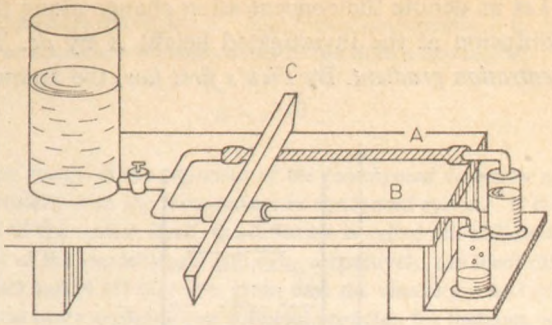

Fig. 4.7. Flow in tubes with elastic and rigid walls

tubes are compressed by the bar C in the shown place at short time intervals (2–3 times per second) during the flow, the flow becomes intermittent; the water flows will differ in the two branches, and in a given time considerably more water will emerge from the end of tube A than from tube B.

This phenomenon can be explained in the following way. Because of its elasticity, the rubber tube expands and contracts periodically, in accordance with the pressure changes. On expansion the kinetic energy of the water is partly transformed into elastic energy, and on contraction part of the elastic energy of the wall is retransformed into kinetic energy. No such energy transformations are produced in the rigid-walled tube, and the lost mechanical energy is transformed into heat as a result of friction.

This experiment is instructive from the viewpoint of blood circulation and supports the physiological observation that the elasticity of the blood vessels is an essential and deciding factor of normal circulation.

4.2. Diffusion and osmosis

4.2.1. Fick's laws

If density or concentration differences exist in some medium, material flow ensues from the higher to the lower densities or concentrations. The spontaneous equalization of the density and concentration differences is called diffusion, which can be observed in gases, fluids and solid states. The phenomenon can be interpreted in terms of molecular thermal motion.

The quantitative discussion of this phenomenon is carried out with reference to Fig. 4.8. For simplicity we assume that the concentration c[1] of the solution in the vessel changes only in one direction (Z), upwards, and that the diffusion takes place

[1] In this case the *concentration of substance* is used, which is the number of moles of the substance in question related to unit volume of the solution. Its dimensions are mol/m^3; mol/l is also used, the latter being the *molarity*.

in this direction. Let us denote the concentration change along the length dz in the direction of the diffusion at the investigated height A by dc. The quantity dc/dz is called the *concentration gradient*. By *Fick's first law*, the amount dv of substance

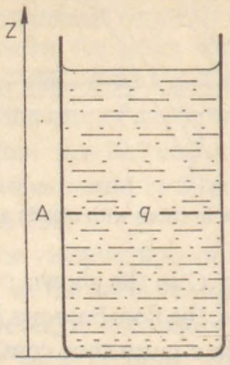

Fig. 4.8. A scheme relating to diffusion

which migrates by diffusion in time dt across the cross-section q is proportional to the concentration gradient, the cross-section and the time, i.e.

$$dv = -Dq\frac{dc}{dz}\,dt, \text{ or } \frac{dv}{dt} = -Dq\frac{dc}{dz} \qquad [4.9]$$

The quotient dv/dt is the *rate of diffusion*. (The negative sign is necessary since dc/dz is a negative quantity.) The proportionality factor D (the *diffusion coefficient*) gives the amount of substance migrating in unit time across unit cross-section in the case of a unit concentration gradient.

Fick's first law holds lastingly only if the concentration distribution does not change in time (stationary diffusion). With *non-stationary diffusion* the concentration of the material studied is a function not only of a place, but also of time. *Fick's second law* holds for this case. Assuming flow only in the Z direction in this case too, the relation

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial z^2} \qquad [4.10]$$

is obtained. Thus, the change in the concentration in time at a given place is proportional to the change in the concentration gradient in space, with the proportionality factor D again denoting the diffusion coefficient. (The partial differentiation is a consequence of the fact that in this case c is a function not only of the z coordinate but also of the time t.)

[4.10] allows the calculation of D if the concentration distribution existing in a given system at a given time is determined. For this purpose [4.10] must be integrated, which can be carried out in special cases, depending on the experimental conditions.

For gases, the diffusion coefficient is approximately proportional to the square root of the absolute temperature, quite similarly to the average thermal velocity of the molecules. In fluids or solids, which are much more close-packed, diffusion is possible because intermolecular gaps (vacan-

cies) always exist which mediate the molecular migration. Consequently, it is understandable that in a given case the diffusion coefficient is proportional to the Boltzmann factor:

$$D \sim e^{-\frac{\varepsilon}{kT}} \qquad [4.11]$$

where ε is *the activation energy of the migration of the investigated molecular species.* According to [4.11], D varies exponentially with the temperature in condensed systems. If the molecules in question are the molecules of the system itself, the diffusion is called self-diffusion and the energies of activation characteristic of the viscosity and diffusion, respectively, are identical.

It follows from [4.3c] and [4.11] that in a given case the viscosity η and diffusion coefficient D are related. For spherical macromolecules or colloidal particles, for instance, regardless of whether the diffusion occurs in gases or fluids, the following equation derived by *Einstein* describes the process to a good approximation:

$$D = \frac{kT}{6\pi\eta r} \qquad [4.12]$$

where r is the radius of the diffusing particle (cf. section 4.6.1). [4.12] is used in several ways, mainly to determine the dimensions and masses (molar mass) of macromolecules.

Table 4.1 lists the values of the diffusion coefficient for a few molecules. Because of the molecular interactions, the value of D depends upon the concentration (the effect may be considerable even at small concentrations in the case of macromolecules), and hence these values are extrapolated to infinite dilution.

Table 4.1

Diffusion coefficients of some compounds in aqueous solution at 20 °C

Compound	D (m²/s)
Glycine	9.5×10^{-10}
Leucyl-glycyl-glycine	4.6×10^{-10}
Ribonuclease	10.2×10^{-11}
Human serum albumin	6.1×10^{-11}
Human haemoglobin	6.8×10^{-11}
Tobacco mosaic virus	3.0×10^{-12}

The tabulated data show that for proteins the value of the diffusion coefficient is in the range 10^{-12}–10^{-10} m²/s, and is more than one order of magnitude smaller than for more simple molecules (e.g. glycine). The differences are due to the differences between the molecular dimensions.

Let us prepare a solution in which the solute molecules are relatively heavy as compared with the solvent molecules. Pour the solution between the two walls of a double-walled glass tube. Heat the inner wall with streaming water vapour, for instance, and keep the outer wall at a lower temperature by cooling. The solute material is observed to collect in greater concentration along the cooler wall than along the warm wall; it flows from a warmer place towards a colder one. This phenomenon, *thermal diffusion,* is even more striking if the tube is kept in a vertical position, for in this case the

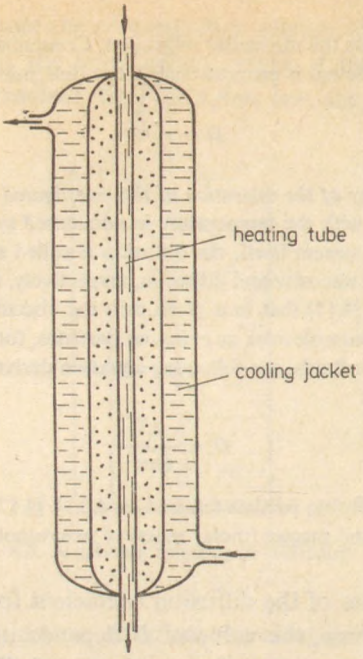

Fig. 4.9. A scheme relating to thermal diffusion

colder and more concentrated solution flows downwards, whereas the diluter and warmer solution moves upwards; a more concentrated solution accumulates in the lower region, and a dilute one in the upper region (Fig. 4.9). Thermal diffusion also occurs in gas mixtures, and one of the most effective methods of isotope separation is based on this process.

4.2.2. Van't Hoff's law

In this section we deal separately with the case of diffusion across a wall, when the wall can be permeated only by some components (*semipermeable wall* or membrane), but is impenetrable for the others. For simplicity, we shall discuss only solutions consisting of two components, and the wall is permeable only for the *solvent*. Immerse a cellophane bag filled with sugar solution into pure water. The bag will swell in a few hours. The compartment outside the bag still contains only water, but the solution within the bag becomes more dilute. Though both the solute and the solvent strive towards uniform distribution, the possibility of this is given for only one component. In our experiment the pressure within the bag steadily increases due to the influx of water, but after some time an equilibrium (dynamic equilibrium) is attained, when the same solvent quantity diffuses into the bag in unit time as is forced out from it by the pressure difference. This phenomenon is called *osmosis*, and the pressure difference which can compensate the influx of the solvent into the bag is the *osmotic pressure*.

Figure 4.10 depicts a simple set-up to measure the osmotic pressure of sugar solutions, for instance. The inner vessel is a porous-walled clay cylinder, which has previously been immersed in copper sulphate, and subsequently in potassium ferrocyanide solution. As a result of this treatment, a copper ferrocyanide film is formed in the pores, which lets through the water, but not the sugar. The sugar solution is poured into the clay cylinder, and the pure water into the outer vessel. After equilibrium has been attained, the osmotic pressure can be read off the mercury manometer.

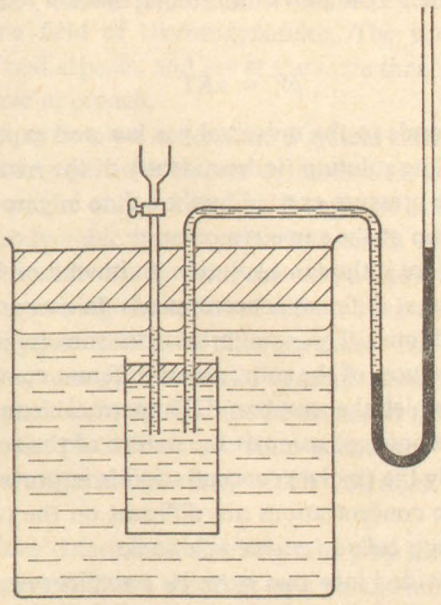

Fig. 4.10. Equipment for measurement of osmotic pressure

The osmosis can be interpreted in terms of the tension decrease of the solutions. The solvent, with the higher tension, is situated on one side of the semipermeable wall, and the solution, with a lower tension, on the other side. The molecules of the solvent can pass through the wall; as a result of the higher tension, more molecules will diffuse through the wall from the pure solvent than in the reverse direction, and hence more solvent will pass into the solution. However, as the pressure within the vessel containing the solution increases due to the solvent migration, the number of molecules diffusing from the solution towards the solvent will also increase. If the pressure becomes large enough, the same number of molecules diffuse towards the pure solvent as in the opposite direction, and dynamic equilibrium is attained between the solution and the solvent. The osmotic pressure (p_{osm}) is the pressure required for the development of dynamic equilibrium at a given temperature. For fairly dilute solution of a non-volatile solute, the experimental results lead to a simple relation, called *van't Hoff's law:*

$$p_{osm} = RTc \qquad [4.13a]$$

where c is the concentration of the solution, and R is the universal gas constant. If c is given in mol/m^3, and R in J/(mol K), p_{osm} is obtained in Pa. Thus, for a dilute solution the osmotic pressure at a given temperature is proportional to the concentration of the solution, and is independent of the materials of the solvent and solute. According to [4.13a] the osmotic pressure of a solution at 20 °C with a concentration of 10 mol/m^3 is 24.3 kPa.

If a solution of volume V contains v mol solute, $c = v/V$. Substitution into [4.13a] yields the equation

$$pV = vRT \qquad [4.13b]$$

[4.13b] formally corresponds to the universal gas law and expresses the fact that the osmotic pressure of a dilute solution (independently of the natures of the solvent and solute) is the same as the pressure exerted by the solute in gaseous form if it occupies the volume of the solution at the same temperature.

Osmosis also takes place if the same solution is situated on both sides of the semipermeable membrane, but at different concentrations. In every case the more concentrated solution will be diluted. The equilibrium pressure is given by the difference between the osmotic pressures of the solutions of different concentrations. Naturally, different substances for which the membrane is impermeable may be dissolved on the two sides. From the viewpoint of osmosis the nature of the solute is immaterial. At a given temperature, only the (molar) concentration is important, and the process of osmosis is induced if the concentrations are different on the two sides. Solutions of equal osmotic pressure are called *isotonic* solutions.

If some solution is divided into two parts by a semipermeable membrane, and a temperature difference is created on the two sides of the membrane, the temperature difference results in a tension difference, which (though the concentrations on the two sides of the membrane are identical) produces a phenomenon very similar to osmosis. The tension on the warmer side increases, and the solvent flows from this into the cooler side; the warmer solution becomes more concentrated, and the cooler more dilute. If the osmosis is due to the effect of a temperature difference, it is called *thermo-osmosis*.

4.3. The first law of thermodynamics

4.3.1. Thermodynamics in general. Basic concepts

Thermodynamics is *the science of equilibria and the processes occurring by energy interactions* in the various phenomena of Nature. The living organism uses the energy of chemical substances taken up from its environment to maintain the processes necessary for its function by using it for work in the cells but affecting the whole organ-

ism. All this is achieved by a long series of chemical and biochemical reactions of nearly constant rate, kept at a constant level and interconnected. The life processes are accompanied by material transport associated with energy transformation, energy exchange, charge flow, and so on, though temporary and local equilibria may also be formed meanwhile. With the aid of the concepts and laws of thermodynamics, reactions, transport processes and equilibria can be quantitatively described, and provide valuable knowledge towards a deeper understanding of the life processes. In the following we shall emphasize only a few basic problems from the broad and continuously expanding field of thermodynamics. The problems to be discussed serve the above-mentioned aspects, and are at the same time necessary for the formation of a thermodynamic approach.

By a thermodynamic system we understand a system consisting of a great number of continuously interacting particles (atoms or molecules, i.e. chemical substances). In actual cases, however, the only substances thought of as belonging to the system are those whose thermodynamic investigation is desired. All other materials which interact with the system under study form the *environment* of this system.

The quantities characterizing the states of thermodynamic systems are *thermodynamic parameters* (briefly parameters) or *state variables*. The best-known parameters are temperature, volume, mass, pressure and concentration, though internal energy, enthalpy, entropy and chemical potential (to be discussed later) are also parameters. The relations between the parameters are state equations. The simplest and best-known equation is the universal gas law, which describes the properties of ideal gases.

Quantities or functions determined unambiguously by the state of the system are called *state functions*, whose change depends only on the initial and final states, but is independent of the path, i.e. the series of intermediate states. All *variables of state are state functions*. However, the heat absorbed or lost or the work done by a system are not state functions, since their quantities may differ for given initial and final states, and consequently these quantities also depend on the path. It follows from the definition that in *cyclic processes* the change in the state functions is zero.

A system without external influence (isolated) is in *thermodynamic* (briefly *dynamic*) *equilibrium* if its parameters do not change, though the change is possible. However, this equilibrium is only a macroscopically static one, since at a molecular level the molecules may be continuously exchanged between the various parts of the system. For instance, at equilibrium between a liquid and its vapour the same number of molecules leave the liquid as return to it from the vapour phase. The state of a non-isolated system may also be unchanged in time, though it exchanges material or energy with its surroundings. However, this is not an equilibrium, but a *stationary state*. This kind of state is attained in continuously functioning reactors, or *between the living cell and its environment* over a not too long time.

Some of the state variables for homogeneous systems in equilibrium are proportional to the extent of the system; these are *extensive* properties, e.g. volume, mass,

electric charge, internal energy, enthalpy, entropy. On the other hand, the properties whose values in case of equilibrium are the same for the parts of the homogeneous system as for the whole system are *intensive* properties. These are for example temperature, pressure, electric potential, chemical potential. The quotients of two extensive properties have also intensive properties; these are the so-called *specific quantities*, e.g. density (mass/volume), or specific electric charge (charge/mass).

4.3.2. Formulation of the first law. Internal energy

It follows from the law of energy conservation that, if the energy supplied to a thermodynamic system or released from the system to its environment is known, the change in the energy content of the system is the algebraic sum of the energies taken up and released by it.[2]

The energies taken up and released can be divided into two groups. One group involves only thermal energy and the second all the various other energy types, and these are taken into account as work done. (The separation is motivated by the special role of thermal energy in Nature, which will be expressed in the second law of thermodynamics.) Let us denote the energy content of a system at the beginning of some process by U_1 and at the end of the process by U_2, while for the sake of brevity the energy change U_2-U_1 is denoted by ΔU. Let us further denote the heat exchanged during the process by Q, and the work done by W. According to the general energy theorem:

$$\Delta U = Q+W \qquad [4.14a]$$

which means *that the change of the energy content in the process is equal to the algebraic sum of the heat exchanged and the work done during the process.* Q is a positive quantity if it denotes the heat taken up by the system, and is negative in the case of a heat loss; further, W is positive if work is done on the system and negative if it is done by the system.

For infinitesimally small changes the following form is used

$$dU = dQ+dW \qquad [4.14b]$$

The changes refer to positive or negative quantities, respectively, if they increase or decrease the energy of the system.

The energy content U has an easily understandable interpretation. U relates to all types of energies which may be possessed by atoms and molecules. Consequently, U includes the kinetic energy due to the continuous, random motion of the atoms and molecules, and the mutual potential energy (bonding energy) of the interacting atoms and molecules. Let us refer to all these energy types overall as the *internal*

[2] The taken-up and released energies are considered to have opposite signs.

energy of the system. Of course, besides this internal energy U also includes the potential and kinetic energies of purely mechanical origin, e.g. the potential energy due to the weight of the system, and the kinetic energy originating from the motion of the system. However, since we deal with systems *at rest* in thermodynamics, and we are generally interested only in the *changes* in the energy content of the system, the purely mechanical energies can be neglected. Thus, the changes in the energy content are due only to the changes in the internal energy of the system, and hence the quantities ΔU and dU in the following relations refer only to the change in the *internal* energy.

[4.14] is the *basic equation of the first law of thermodynamics*. If it is added that the *internal energy is a state function*, the formulation of the first law of thermodynamics is complete.[3]

The overall importance of the internal energy is obvious from the above discussion. Nevertheless, it is worthwile repeating that the concept of internal energy is important in all processes in which the mean kinetic energy or (as a result of rearrangement) the mutual potential energy of the atoms forming a system become changed. Such changes occur in processes accompanied by temperature change, phase transitions, chemical (biochemical) reactions, and so on. It follows that a close connection exists between the internal energy and the specific heats, the heats of transition, and the heats of reaction. A knowledge of these quantities allows the determination of the internal energy change of a system in some given process (cf. section 4.3.3).

4.3.3. Examples of application of the first law. Addenda

1. Specific heat and internal energy. Volumetric work. If the temperature of a system, e.g. a gas, is changed, its internal energy also changes. The quantitative considerations are discussed for two cases:

(a) Let us heat the gas without changing its volume *(isochoric or isosteric process)*. According to the first law, since no work has been done, the change in the internal energy is equal to the heat supplied, i.e.

$$\Delta U = Q_V, \quad \text{or} \quad \Delta U = c_V m \Delta T \qquad [4.15]$$

where c_V is the specific heat of the gas at constant volume, m is its mass, and ΔT denotes the temperature change. (The index V refers to the constant volume in the case of supplied heat too.)

[3] In themselves, the exchanged heat and the work done generally depend not only on the initial and final states, but also on the means of change. The symbol d usually denotes only the change in the state functions, while the changes in the other functions are denoted by the letters D or δ. In this book we disregard this notation and the infinitesimally small changes are uniformly denoted by the symbol d.

(b) Let us heat the gas at constant pressure *(isobaric process)*. On heating, the gas expands and work is done against the external (for instance the atmospheric) pressure. If the volume change is denoted by ΔV and the pressure by p, the work done is given by

$$W = -p\Delta V \qquad [4.16a]$$

and in this case the change in the internal energy of the gas, according to the first law, will be

$$U = Q_p - p\Delta V \quad \text{and} \quad U = c_p m\Delta T - p\Delta V \qquad [4.16b]$$

where the index p refers to the constant pressure. The content of [4.16b] (writing the work on the left-hand side of the equation) can be formulated so that the heat supplied only partly increases the internal energy, the other part being converted to work.

The above discussion reveals that two kinds of specific heat exist: specific heat at constant volume (c_V) or at constant pressure (c_p). For solids and fluids the difference between the two specific heats can usually be neglected, for the difference due to the volume change caused by heating and the related work is negligibly small. However, for gases the value of c_p/c_V is between 1.2 and 1.7.

In the present case [4.16a] has been used for gas expansion though it yields the volumetric work correctly for compression too. Consider that, on expansion, i.e. in the case of positive ΔV, the work is done by the system, as given by [4.16a], and is conventionally a negative quantity. On compression, on the other hand, i.e. in the case of negative ΔV, the work is done on the system, again as in [4.16a], and is conventionally a positive quantity.

2. Enthalpy. In practice the different physical and chemical processes take place at constant pressure, usually under atmospheric conditions. This statement is also valid for living processes.

Processes at constant pressure can be described in a simple way, if a new state function, called *enthalpy* (H) is introduced:

$$H = U + pV \qquad [4.17a]$$

where U is the internal energy of the system, V is the volume and p the pressure of the system in *equilibrium* at a given volume and temperature. It follows from the definition that the equilibrium pressure is always equal to the external pressure (for instance the atmospheric pressure) acting on the system. For processes at constant pressure the change in the enthalpy due to the changes in U and V will be

$$H = \Delta U + p\Delta V \quad \text{and} \quad dH = dU + pdV \qquad [4.17b]$$

respectively. Thus, the enthalpy change is given by the sum of the internal energy change and the volumetric work. This allows the change in the internal energy and the volumetric work in *isobaric* processes to be expressed as the change in a single quantity. For instance, after appropriate rearrangement [4.16b] can be written in

the form $\Delta H = Q_p$. It is generally true that in *isobaric* processes, where there is only volumetric work, the exchanged heat is equal to the change in the enthalpy:

$$\Delta H = Q_p \qquad [4.18]$$

and in those *isosteric* processes where, other than the naturally missing volumetric work, no work is involved, the exchanged heat is equal to the change in the internal energy

$$\Delta U = Q_V \qquad [4.19]$$

3. Transition heat and enthalpy (internal energy). If a solid is melted at constant temperature and the small volume change on melting is disregarded, the total heat input will be used to increase the internal energy. Consequently, we may write a relation similar to [4.19], where the heat input (and consequently the change in the internal energy) can be expressed with the melting heat. On evaporation, however, the volume increase resulting from the transformation into vapour is accompanied by considerable work, which must be taken into account. In this case the energy relations are described by [4.18]. The heat uptake which can be expressed with the heat of evaporation is equal to the enthalpy change. If the volumetric work is subtracted from this, the internal energy change due to evaporation is obtained. The first law can be applied in a similar way to processes in the opposite direction, i.e. to freezing and condensation, which are accompanied by a decrease in the internal energy, i.e. a decrease in the enthalpy. Our findings accordingly hold for all types of phase transitions.

Measurements indicate that a work of 40.7 kJ is required for the evaporation of 1 mol (18 g) water at 100 °C and a pressure of 101 kPa. This is the value of the enthalpy increase in this case. Part of this work is used to perform the volumetric work against the atmospheric pressure; its value is

$$W = -p(V_{\text{vapour}} - V_{\text{water}}) \approx -pV_{\text{vapour}}$$

However, according to the universal gas law

$$pV_{\text{vapour}} = \nu RT$$

Since $R = 8.31$ J/(mol K) and in this case $\nu = 1$ mol and $T = 373.16$ K, in our example we have

$$W \approx -3.1 \text{ kJ}$$

and the increase in the internal energy is only 37.6 kJ. Consequently, approximately 0.4 eV per molecule is required to vaporize water at 100 °C.

4. Reaction heats and enthalpy (internal energy). An understanding of chemical reactions requires a knowledge of the heat liberated or taken up during the reaction at constant temperature (isothermal process). The amounts of substances participating in a reaction are usually given in mol units, and consequently the liberated or absorbed heat is related to 1 mol of substances; this is called the *reaction heat.*

In isothermal–isosteric reactions the reaction heat is measured at constant volume, and in isothermal–isobaric reactions at constant pressure. In the first case the change in the internal energy (also related to one mol) is equal to the reaction heat Q_V, i.e.

$$\Delta U = Q_V \qquad [4.20]$$

while in the latter case the heat of reaction is partly required for work associated with volume changes, and thus the reaction heat Q_p is equal to the change in the enthalpy and not the internal energy, i.e.

$$\Delta H = Q_p \qquad [4.21]$$

In reactions in the liquid or solid phase the volumetric work can be neglected and [4.20] can be accepted as holding. In reactions in the gaseous phase, however, when the vo umetric work can generally not be neglected, calculations should be carried out with [4.21].

Measurements on the conversion of 1 mol dextrose to 2 mol ethyl alcohol and 2 mol carbon dioxide at 25 °C and 101 kPa pressure show that 71.2 kJ heat is liberated. The enthalpy change for 1 mol dextrose is thus $\Delta H = -71.2$ kJ. The change in the internal energy can be determined by considering the volumetric work done during the process, which is obtained mainly from the formation of 2 mol gaseous carbon dioxide. This work can be calculated in a similar way as for the evaporation of water in the previous example. The result, i.e. the work done by the system, is $-p\Delta V = -5$ kJ. Thus, the change in the internal energy is $\Delta U = \Delta H - p\Delta V = -76.2$ kJ. This means that rearrangement of the atoms of a dextrose molecule into two ethyl alcohol and two carbon dioxide molecules results in a more stable configuration than the original one, as characterized by the liberation of approximately 0.8 eV.

5. Determination of the enthalpy (internal energy). On the basis of the relations derived in the previous sections, the *changes* in the enthalpy and the internal energy can be determined if the specific heats at constant temperature and constant volume and (if phase transformations or chemical reactions take place during the process) also the heats of transformation and heats of chemical reaction are known.

If the internal energy at absolute zero temperature were known, the absolute values of the internal energy and enthalpy in different states could be determined. This is possible for only a few substances, but this is not a problem of practical importance, for we are generally interested merely in the changes in enthalpy (internal energy) in various processes or transformations. For the sake of uniformity, however, international conventions regulate the initial values and states to be considered when determining the changes. Accordingly, the enthalpy (internal energy) is fixed so that the enthalpies (internal energies) of the *chemical elements* at 25 °C and 101 kPa (in the state in which they are stable) are considered to be zero. From this it follows that the enthalpies (internal energies) of the *chemical compounds* at 25 °C and 101 kPa are equal to their heats of formation at constant pressure (constant volume). This heat of formation (or more exactly its value related to one mol) is called the *standard heat of formation* or *standard enthalpy*, denoted by H° or ΔH°. Tab e 4.2 lists the standard enthalpies of some substances.

Table 4.2

Standard enthalpies (H°) of some substances

Element or compound	State	H° (kJ/mol)
H_2	g	0.0
O_2	g	0.0
C (graphite)	s	0.0
H_2O	l	−286.0
H_2O	g	−242.0
CO_2	g	−394.0
Acetic acid	l	−487.4
Lactic acid	l	−677.0
Ethyl alcohol	l	−278.0
Glycerine	l	−666.6
Glucose	s	−1280.1

g=gas or vapour; l=liquid; s=solid

6. The Hess theorem. According to the first law, neither a change in internal energy nor a change in enthalpy depend upon the path, and consequently the heat of reaction does not depend on the intermediate states, but only on the initial and final states. Hess discovered this theorem (referred to in chemistry as the *Hess theorem*) before the general energy theorem was recognized. The Hess theorem is a special case of the first law of thermodynamics.

The Hess theorem is of great practical importance, since it is not necessary to measure the heat of reaction of all reactions, for instance. By means of this theorem the heat of reaction can be *computed* from the heats of formation of the interacting and the produced substances. The heats of formation of a great variety of compounds can be found in tables published in the literature. If the heat of formation of a compound cannot be measured directly, it can be determined from the heat of one of its known reactions (in the case of organic substances, usually from the heat of combustion). The nutritional value of food is usually measured by its heat of combustion. This is possible because the heat liberated from a given amount of substance is the same when an organic compound is either burnt into carbon dioxide and water, or is oxidized within the organism in several steps (through complex processes) to the final products carbon dioxide and water.

4.4. The second law of thermodynamics

4.4.1. Formulation of the second law. A statistical interpretation of entropy

According to the first law, in Nature only processes in which the total energy remains unchanged take place. In reality, however, not all processes, being possible on the basis of the first law, occur. For instance, let us drop a stone from some height. On inelastic collision the mechanical energy of the stone will be transformed into heat. According to the first law, a process with opposite direction might also be possible, in which the heat is retransformed into mechanical energy, and the stone and its surroundings regain their initial position. However, this kind of process is never encountered. If a strong base reacts to a strong acid, a salt is produced spontaneously. The opposite process, however, never occurs; a salt never decomposes spontaneously into a base and an acid. The pressure, temperature and concentration differences in systems without external influences tend to become equalized, and never increase. The second law of thermodynamics deals with this problem, i.e. with the direction of spontaneous processes.

The factors determining the direction of spontaneous processes are related to *heat*, and in every case can be ascribed to the universal experience that *heat always flows spontaneously from a warmer to a cooler body*. Various, equivalent definitions of the second law are known, though perhaps the above is the simplest of all; its content can readily be fo lowed as concerns atomic or molecular aspects. Heat is a form of energy, which has its origin in the random motion of atoms or molecules. When two systems come into contact, for instance, that in which the mean kinetic energy of the molecules is higher can transfer heat to the other. This seems to be quite natural. If billiard balls collide, it is more probable that the ball with higher energy will transfer some of its energy to the ball with lower energy, and not vice versa; the latter process may also occur, but with much lower probability. The situation is quite similar in the case of molecular collisions. In principle it may be possible that the molecules of the warmer body gain energy from the molecules of the colder one, i.e. the warmer body obtains energy from the colder one, but this is so improbable that it does not occur spontaneously in practice. This type of process can take place only with some external aid. (Consider e.g. the refrigerator.) According to this concept, the *second law of thermodynamics is a statistical one in character and simply expresses the fact that the thermodynamic processes progress spontaneously towards the more probable state*. In principle the opposite processes may also occur but this is not probable spontaneously in practice. Processes in the opposite direction can occur in reality only if they are accompanied by some other changes. In an isolated system, processes of any kind can be observed only until the system reaches its most probable state; when this has been attained, the system has reached thermodynamic equilibrium, from which it can be displaced only by some external effect.

Entropy. The quantitative formulation of the first law was made possible by the use of a state function, the internal energy. The quantitative definition of the second law requires a new state function, *entropy*. Entropy can be expressed by probabilities, more exactly as will be seen below, by *thermodynamic probabilities* associated with the individual states. According to Boltzmann, the entropy S of a system in a given state is proportional to the logarithm of the thermodynamic probability (w) associated with this state. If natural logarithms are used the proportionality factor k is the Boltzmann constant; thus, we have

$$S = k \ln w \qquad [4.22a]$$

Let us denote the thermodynamic probability at the beginning of the process by w_A and the entropy by S_A, and in the final state let the probability be w_B and the entropy S_B. With these notations the entropy change will be expressed by the relation

$$S_B - S_A = k \ln \frac{w_B}{w_A} \qquad [4.22b]$$

It is generally true that in any isolated system the processes progress in the direction of an increase in the thermodynamic probability and hence in the entropy. The thermodynamic equilibrium is characterized by the maximum of the thermodynamic probability, and by that of the entropy.

The determination of the thermodynamic probability is illustrated by a simple example. Let us consider an isolated vessel which contains a gas of 4 identical point-like molecules denoted by the letters *a*, *b*, *c* and *d*. We now examine the possible distribution of these molecules in the two halves (cells I and II) of the vessel. The possibilities are summarized in column 3 of Table 4.3. Altogether 16 random distributions or *microstates* are conceivable. However, measurement of the various physical properties (e.g. the density) reveals no difference between the various microstates, i.e. from a macroscopic point of view e.g. the 4 various microstates in the 2nd row are identical. The 6 microstates in the 3rd row are different from the states in the 2nd row, but

Table 4.3

Example to illustrate thermodynamic probabilty

Macro-states	Molecules in cell I		Number of micro-states
	number	notation	
A	4	abcd	1
B	3	abc, abd, acd, bcd	4
C	2	ab, ac, ad, bc, bd, cd	6
D	1	a, b, c, d	4
E	0	—	1

identical with each other. Similarly, the microstates in the 4th row, though differing from the states in the other rows, are again found to be identical macroscopically. In reality only five different states can be observed; these *macrostates* are denoted by capital letters in column 1. The number of microstates relating to the individual macrostates are given in column 4. If it is assumed that the individual microstates are equally probable, it is quite clear that the most probable macrostate will be the state associated with the highest number of microstates. In the above case, therefore, the most probable state is state C (i.e. uniform distribution), and states A and E are the least probable, the distributions there being the least uniform. *The thermodynamic probability of a macrostate is characterized by the number of microstates associated with the respective macrostate.*

The thermodynamic (statistical) probability is not identical with the mathematical probability. For calculation of mathematical probability associated with some macrostate, the quotient of the numbers of favourable and possible cases is needed. The number of favourable cases of a given macrostate is given by the number of the *associated* microstates, and the number of possible cases (for any macrostate) is equal to the number of *all* possible microstates. For instance, the thermodynamic probability associated with macrostate B in the above example is 4, while the mathematical probability is 4/16; the corresponding quantities in case C are 6 and 6/16.

In practice one usually deals with the *changes of state* of a system. In this context the *quotient* of the probabilities is considered as in [4.22b]. It follows that, because of the quotient formation in the case of changes of states, the thermodynamic probabilities yield the same results as the mathematical probabilities.

In the previous example the possibilities of the spatial distribution have been investigated. Similar calculations could be carried out with respect to the distribution of the kinetic energy. The result would show in this case too that the number of microstates is highest when the kinetic energy, i.e. the temperature, is identical in the cells.

If the system consists of multiatomic molecules, the motion of the molecules includes their rotations and the vibrations of the atoms in the molecule. All these motions must be taken into consideration in a calculation of the total entropy of the system. It follows that the entropy is the sum of several terms. All processes increasing the molecular mobility also increase the entropy of the respective system. Examples of such processes are melting, evaporation, dissolution, diffusion, the expansion of gases, etc. The loosening or cleavage of the bonds between the atoms within the molecules, e.g. molecular dissociation, results in an increase of entropy. In contrast, all processes leading to a strengthening of the atomic bonds or limiting the molecular motion decrease the entropy. Such processes are freezing, condensation and the formation of molecules from atoms or atomic groups.

An increase in the thermodynamic probability can be regarded as associated with a decreased ordering, and vice versa. For instance, the uniform distribution of gases in a given volume is viewed as a lower degree of ordering than their distribution in only part of the volume. For this reason it may be stated that *thermodynamic*

processes in a system without external influences proceed in the direction of lower ordering.

It follows from the statistical meaning of the second law that the equilibrium state of a thermodynamic system is its most probable state; however, this may allow some local and transient fluctuations. For instance, temperature, pressure, density and concentration fluctuations may be expected. Such phenomena can be observed, and are experimental proof of the molecular heat theory. As an example, the blue colour of the sky may be explained by the irregular, slight density fluctuations of the air. Brownian motion is also a fluctuation phenomenon.

In practice, entropy is usually determined via directly measurable quantities and not by calculating the thermodynamic probability. Before the phenomenological definition of entropy related to macroscopic quantities is given, a more profound analysis of the thermodynamic processes is necessary; this is performed in the next section.

4.4.2. Thermodynamically reversible and irreversible processes

Any process is said to be thermodynamically *reversible* if the system participating in the process returns to its initial state so that no change remains even in its surroundings. In the opposite case the process is *irreversible.*

According to the second law, which is derived empirically, no *strictly reversible* process exists in Nature. In reality, processes proceed spontaneously in only one direction, and some external influence is necessary for the opposite direction to be taken. Though this external aid may cause the system to return to its initial state, some changes may be left over in the surroundings. The irreversible character of a process is related to heat phenomena, which are always present in practice. Mechanical, electric and optical processes would be reversible if they were not accompanied by heat phenomena. For instance, the motion of a pendulum would be reversible if there were no friction, and similarly the motion of a falling and rebounding ball would be reversible if a perfectly elastic collision existed. Further, an electromagnetic field would propagate reversibly without the absorption phenomena associated with this propagation. Diffusion, evaporation, heat conduction, the volume change of gases, and chemical processes are typically irreversible processes. Though real processes are never reversible, *all changes of state can be conceived of as reversible.* The study of these idealized changes of states is important in practice, since the resulting knowledge provides an insight into the actual processes. The phenomenological definition of entropy is also related to reversible processes.

It is relatively easy to conceive the reversibility of mechanical, electric or optical processes. For this reason some examples of typically irreversible processes will be dealt with in the following. For simplicity, physical processes will be treated, though we should like to emphasize that any change of state, and consequently any chemical

reaction, may be performed reversibly. The processes associated with heat phenomena could be reversible only if they proceeded *infinitesimally slowly in several steps through individual equilibrium states, i.e. quasi-statically.*

1. As a first example, let us consider the *isothermal volume change* of a gas in a cylinder closed by a piston moving without friction. To begin with we examine the expansion, and subsequently the compression. For this purpose we calculate the work done by an amount v of gas at temperature T while its volume changes from V_A to V_B and its pressure from p_A to p_B (Fig. 4.11*a*). The equilibrium pressure of the gas at various volumes is given by the universal gas law and is depicted by the solid line in Fig. 4.11*a* (isotherm). First let us decrease the gas pressure to p_1. As a result, the volume of the gas will undergo the change ΔV_1. The work done during this process will be $-p_1 \Delta V_1$. Next, the external pressure is decreased to p_2, when the volume change will be ΔV_2. The work done in the second step will thus be $-p_2 \Delta V_2$. If the expansion is continued for n steps, the total work done is given by the sum

$$W_n = -\sum_1^n p_i \Delta V_i \qquad [4.23a]$$

which is represented in Fig. 4.11*a* by the sum of the areas of the dot-filled rectangles.

A similar method may be used to calculate the work done on the isothermal compression of the gas. Whereas pressures lower than the equilibrium pressures are applied on expansion, pressures larger than the equilibrium pressures should be applied on compression (Fig. 4.11*b*). In the second case the various quantities are denoted by the same letters as in the first case, but with the addition of commas. Consequently, the total work done on compression will be

$$W'_n = -\sum_1^n p'_i \Delta V'_i \qquad [4.23b]$$

which is shown by the sum of the dot-filled rectangles in Fig. 4.11*b*.

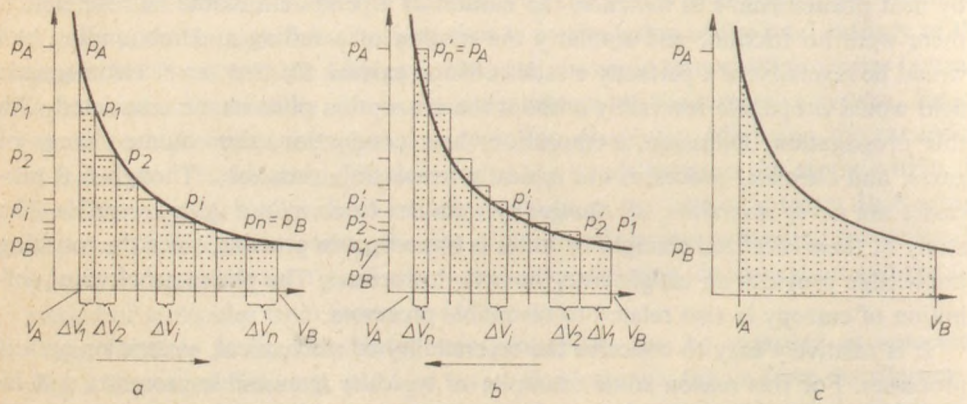

Fig. 4.11. Diagrams relating to the isothermal change of state of gases
a: irreversible expansion; *b:* irreversible compression; *c:* reversible change of state

On the isothermal expansion or contraction of gases, heat exchange too takes place between the gas and its surroundings. On expansion the gas takes up as much heat as required for the work to be done, and on compression as much heat is released into the surroundings as the work done on the gas. Thus, [4.23a] and [4.23b] and the sums of the rectangle areas yield information about the heat exchange too.

From the above discussion it is clear that, at the end of a cycle consisting of an isothermal expansion and a subsequent isothermal compression, only the gas returns to its initial state, but some change remains in the surroundings: *mechanical work is lost, and heat equivalent to the lost work is gained.* Hence, the process is irreversible.

It may be concluded from the above considerations that, with an increase in the number of expansion and compression steps, the work gained on expansion can be increased and the work necessary for compression can be decreased, and if $n \to \infty$ the two works approach *a common value.* This common value is depicted by the area under the isotherm in Fig. 4.11*c*. The heats released and taken up also approach the same value, which is again the area under the curve. Thus, if the volume changes could proceed in infinitesimally small steps, i.e. if we could deviate from every equilibrium state by only an infinitesimally small degree, at the end of the cycle the algebraic sums of the work done and of the heat exchanged would both be zero. In the event of such a quasi-static process, no change would remain in the surroundings and the process would be reversible.

The work associated with the reversible path can be computed by integration. For the work W_{AB} done on reversible expansion the following equations hold:

$$W_{AB} = \lim_{n \to \infty} W_n = -\int_{V_A}^{V_B} p dV \qquad [4.23c]$$

However, according to the universal gas law, $p = vRT/V$, and the product vRT is constant, so that

$$W_{AB} = -vRT \ln \frac{V_B}{V_A} \qquad [4.23d]$$

Instead of [4.23d], we may write

$$W_{AB} = -vRT \ln \frac{p_A}{p_B} \qquad [4.23e]$$

where the equality $V_B/V_A = p_A/p_B$ has been taken into consideration.

From the previous relations the heat Q_{AB} exchanged on reversible expansion too can be obtained directly:

$$Q_{AB} = vRT \ln \frac{V_B}{V_A}, \quad Q_{AB} = vRT \ln \frac{p_A}{p_B} \qquad [4.23f]$$

The work done and the heat exchanged on compression can be calculated in the same way. The results differ only in sign.

On the basis of the discussed example, the *following general statements can be made:*

(a) In the course of a change of state, the work done *by* a system is maximum or (in the case of opposite direction) if done *on* the system it is minimum if the system transforms reversibly from one state into the other. The maximum work done is always equal to the minimum work taken up by the system.

(b) In real, i.e. irreversible cyclic processes, a certain amount of work is always lost, and instead heat develops.

2. In the next example *isothermal diffusion (isothermal mixing)* is dealt with. Consider a case when, at the beginning of the process, the lower half of a vessel is filled with an ideal solution, with above it a pure solvent (Fig. 4.12). The volume of the solute in the initial state is V_A, while in the final state it is V_B. Let us denote the initial concentration by c_A and the final concentration by c_B. The system can pass from its initial to its final state via various paths. It will be shown that *in a quasi-static osmotic process the diffusion too may proceed reversibly.* For this purpose imagine the pure solvent to be separated from the solution by means of a piston which is permeable only for the pure solvent. The osmotic pressure may be compensated if an appropriate weight is placed on the balance. Subsequently, we begin to decrease

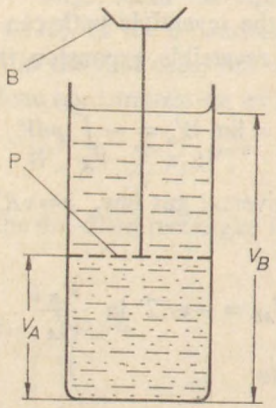

Fig. 4.12. Diagram relating to reversible diffusion
P: piston made of semipermeable material; *B:* balance

the force acting on the balance in infinitesimally small steps. During this process the piston gradually rises while the solution becomes continuously more dilute. The pressure acting on the balance at every moment is in quasi-equilibrium with the osmotic pressure. The work done by the system during the dilution (W_{AB}) can be calculated similarly as in the isothermal expansion of gases. From the analogy between the universal gas law and the van't Hoff law, the final result is similar to [4.23e], but in this

case v denotes the amount of solute. Consequently

$$W_{AB} = -vRT \ln \frac{V_B}{V_A} \qquad [4.24a]$$

and since $c_A = v/V_A$ and $c_B = v/V_B$, instead of [4.24a] we may write

$$W_{AB} = -vRT \ln \frac{c_A}{c_B} \qquad [4.24b]$$

The dilution accompanied by the work is associated with an exchange of heat, which yields an expression similar to [4.23f].

Analogously, processes opposite to diffusion or dilution, i.e. reversible concentration, may also be conceived. The solute distributed in the volume V_B may be collected in the volume V_A if the pressure on the balance is always larger than the actual osmotic pressure, and if it is finally equal to the pressure associated with V_A. With a quasi-static concentration increase the work done on the system is the same as the work done by it on dilution. This is valid for the exchanged heat too. Thus, by a cyclic process consisting of diffusion and subsequent concentration, the system can finally be brought back into its initial state so that no change remains in its surroundings.

3. We next consider an example where no work is done and only *heat exchange accompanied by temperature change* takes place. Let us warm 240 g water from 20 to 21 °C. This is done in principle by bringing the water into contact with some heat reservoir at appropriate temperature. If the temperature of the heat reservoir is 21 °C and the reservoir can be regarded as infinitely large, the heating may be carried out in one step, and the system takes up 1 kJ. However, the heating can also be carried out by the use of not one, but a hundred heat reservoirs whose temperatures rise progressively from 20 to 21 °C in 1/100 °C steps, so that the water is brought into contact with reservoirs of gradually increasing temperature. The higher the number of heat reservoirs used and the finer the gradual steps, the more closely the situation approaches reversible heating via the equilibrium states. The reverse process, i.e. reversible cooling, may be conceived in a similar way.

It should be observed that, if the water has been heated in 1/100 °C steps and cooled in the same way, the remaining change consists of the loss of 1/100 kJ heat by the reservoir at 21 °C while the reservoir at 20 °C gains the same amount of heat, which means that heat has been transferred from the reservoir of higher to the reservoir of lower temperature. With even smaller steps, the change will decrease. In the limiting case, i.e. in the event of a reversible process, no change remains.

4.4.3. Phenomenological definition of entropy

1. Definition. A development of the concept of entropy is beyond the scope of this book. We merely give its definition. Let a thermodynamic system be transferred from a state A into a state B. From a given initial state, the system can be transferred

into its final state via various paths. In reality every path is irreversible, though a given transformation of state might be conceived of as reversible. *The phenomenological definition of entropy is obtained by considering reversible processes.* Let S_A be the entropy of the system in the A state, and S_B that in the B state. The entropy change in the process $A \rightarrow B$ is given by the relation

$$S_B - S_A = \int_A^B \frac{dQ_{\text{rev}}}{T} \qquad [4.25a]$$

The quantity dQ_{rev} denotes the heat exchanged between the system and its surroundings in an infinitesimal change of state at absolute temperature T, when the change of state is reversible (the subscript rev refers to this). Instead of [4.25a], the entropy can also be defined by its infinitesimally small change

$$dS = \frac{dQ_{\text{rev}}}{T} \qquad [4.25b]$$

If the system takes up heat in the course of the reversible change of state, its entropy increases, whereas a heat loss results in a decrease in entropy. It is again emphasized that entropy is a state function, whose value depends only upon the initial and final states, and is independent of the path along which the transformation of the state actually occurs. Its dimension is J/K.

At first sight the statistical and phenomenological interpretations of entropy differ considerably: cf. [4.22b] and [4.25a]. However, Boltzmann showed the equivalence of these two relations, as long ago as the second half of the 19th century, and in doing so connected phenomenological thermodynamics with molecular and statistical theory. The equivalence of the two relations is presented here in a simple case.

Consider the change of state of ν mol of an ideal gas in a volume fraction V_A of a vessel when the gas expands at a constant temperature T until it fills the total volume V_B of the vessel. From [4.25a], the entropy change $S_B - S_A$ associated with this change of state is obtained by determining the heat Q_{AB} taken up during the *reversible* expansion and dividing it by the temperature T. From [4.23f], we have

$$S_B - S_A = \int_A^B \frac{dQ_{\text{rev}}}{T} = \frac{1}{T} \int_A^B dQ_{\text{rev}} = \frac{Q_{AB}}{T} = \nu R \ln \frac{V_B}{V_A}$$

and by further transformation

$$S_B - S_A = Nk \ln \frac{V_B}{V_A} = k \ln \left(\frac{V_B}{V_A} \right)^N$$

where $N = \nu L = \nu R/k$ is the number of gas molecules. On the other hand, from the statistical interpretation and [4.22b] we have

$$S_B - S_A = k \ln \frac{w_B}{w_A}$$

It must be proved that the relations derived from the two types of interpretation can be transformed into each other. This task is equivalent to proving the equality

$$\frac{w_B}{w_A} = \left(\frac{V_B}{V_A} \right)^N$$

For this purpose let us investigate more closely the meaning of the quotient w_B/w_A; this tells how many times the probability of uniform occupation of the volume V_B is greater than the probability that only the smaller volume V_A is filled. It is readily seen that this quotient is equal to the power expression on the right-hand side. Since the equation contains quotients, mathematical probabilities may be used instead of thermodynamic probabilities in the calculations. The mathematical probability of finding the N molecules in the total volume V_B is equal to 1, i.e. certainty. On the other hand, the probability of finding N molecules in the volume V_A smaller than V_B is V_A/V_B for the case $N=1$, $(V_A/V_B)^2$ for $N=2$, and $(V_A/V_B)^N$ for the case $N=N$. Thus, the above equivalence is proved.

Our example also shows that, in the statistical interpretation of entropy, calculations cannot be made simply with the thermodynamic probability; instead, its logarithm is required. In other words, in place of [4.22a] one cannot simply write a proportionality between the entropy and the thermodynamic probability, though this would also express the basic requirement that the two quantities should change in the same sense. The statistical interpretation leads to a result identical with the phenomenological definition only if instead of the thermodynamic probability its logarithm is taken into account. The essence of the logarithmic relation becomes even clearer in the following consideration.

Let us take any thermodynamic system in an arbitrary macrostate. Let the thermodynamic probability of the macrostate be w, and let S denote the entropy of the system. Let us in imagination divide our system into two parts, and denote the thermodynamic probabilities and entropies of the subsystems by w_1 and w_2 and by S_1 and S_2, respectively. The following facts must be considered:

(a) Entropy is an extensive quantity, i.e. the entropy of the total system is equal to the sum of the entropies of the subsystems:

$$S = S_1 + S_2$$

(b) From probability theory the microstates associated with all the macrostates of the total system can be produced by combining every microstate in one subsystem in every possible way with the corresponding microstates in the other subsystem. If the number of microstates in one subsystem is w_1 and that in the other subsystem is w_2, the number of microstates in the total system is given by the equation

$$w = w_1 w_2$$

Consequently, the relation between the entropy and the thermodynamic probabilities must be of a form which satisfies (a) with the use of (b). It is immediately seen that a logarithmic relation corresponding to [4.22a] satisfies this requirement.

2. Examples. Standard entropy. We shall now calculate the change of entropy at the melting of 1 kg ice at $T=273$ K and 101 kPa pressure. Since the process takes place at constant temperature, the quantity $1/T$ can be put before the integral sign in [4.25a], and the summation can be carried out only for the heat uptake during the melting. Measurements show this heat to be 335 kJ, and the change of entropy will therefore be 1.23 kJ/K.

Let us calculate the change of entropy (S_B–S_A) when 1 kg water is heated at constant pressure from T_A=273 K to T_B=323 K. Since $dQ_{rev}=cm\Delta T$ in this case, where c denotes the specific heat of water and m is its mass, both factors can be written before the integral sign and we have

$$S_B - S_A = cm \int_{T_A}^{T_B} \frac{dT}{T} \quad \text{and} \quad S_B - S_A = cm \ln \frac{T_B}{T_A} \qquad [4.25c]$$

The result of the calculation is 703 J/K.

The entropy changes associated with the temperature change or phase transition of other substances (elements or compounds) can be determined similarly. Only a knowledge of the specific heat and the heat of transformation is necessary for the calculations. In practice, mainly the change of entropy is involved, though determination of *the absolute value of the entropy* is also possible. (This possibility does not generally exist with the internal energy or enthalpy; cf. section 4.3.3.) From considerations not discussed here, *the entropy of every chemically uniform substance is zero at absolute zero temperature*. Starting from this, with the aid of the specific heats and the heats of phase transitions the absolute values of the entropy of individual substances can be determined for any temperature. The entropy values associated with 25 °C and 101 kPa pressure are usually given for 1 mol amount of substance. This value of the entropy is called the *standard entropy* (Table 4.4).

Table 4.4

Standard entropies (S°) of some substances

Element or compound	State	S° (J/mol · K)
H_2	g	130.6
O_2	g	205.2
C (graphite)	s	5.9
H_2O	l	69.9
H_2O	g	188.8
CO_2	g	214.0
Acetic acid	l	159.9
Lactic acid	l	192.2
Ethyl alcohol	l	160.8
Glycerine	l	208.1
Glucose	s	212.3

g=gas or vapour; l=liquid; s=solid

4.4.4. Direction and equilibrium of adiabatic processes

In connection with the statistical interpretation of entropy, it is easy to see (cf. section 4.4.1) that in an isolated system real processes proceed in the direction of increase in the entropy until it reaches the maximum possible value for the given system. However, experience reveals that this also holds under conditions where the system is not strictly isolated. The finding is also true for adiabatic processes, since the system must be isolated from its surroundings as concerns heat exchange, but not necessarily as concerns the work done. The processes in adiabatic systems can be characterized briefly by the formula

$$dS \geqq 0 \qquad [4.26]$$

where the sign of inequality relates to adiabatic processes, and the equality sign to thermodynamic equilibrium.

The validity of [4.26] can be demonstrated by a simple example. Consider an adiabatically closed vessel whose volume is divided into two parts by a wall made of some heat-conducting material. One part of the volume is filled with a gas at temperature T_1 and the other part with the same gas at temperature T_2. Let us now follow the initial process of temperature equalization. It will be shown that this process is characterized by [4.26].

Since the system is adiabatic, the algebraic sum of the heat exchange between the two volume parts is zero, i.e.

$$dQ_1 + dQ_2 = 0, \quad \text{or} \quad dQ_2 = -dQ_1$$

The entropy change (dS) in the course of this process is the sum of the entropy changes (dS_1 and dS_2) of the component systems, i.e.

$$dS = dS_1 + dS_2 = \frac{dQ_1}{T_1} + \frac{dQ_2}{T_2} = dQ_1 \left(\frac{1}{T_1} - \frac{1}{T_2} \right)$$

The following conclusions can be drawn from the equation:

(a) if $T_1 > T_2$, $dQ_1 < 0$, and consequently $dS > 0$;

(b) if $T_1 < T_2$, $dQ_1 > 0$, and consequently $dS > 0$;

(c) if $T_1 = T_2$ equilibrium exists and $dS = 0$.

The conclusions thus prove [4.26].

Of course, in non-adiabatic systems processes may also take place in which the entropy decreases. For instance, the entropy of liquids freezing at constant temperature decreases, as does the entropy of living organisms during development. [4.26] holds only if, besides the solidifying liquid, the bodies taking up the heat liberated on freezing are also taken into consideration; in the case of living organisms, attention should be paid to the food consumed, the metabolic end-products, etc. In the isolated (or only adiabatic) systems formed in this way, processes involving both entropy increases and entropy decreases occur, and only the entropy of the overall system increases.

4.4.5. Direction and equilibrium of isothermal processes. Helmholtz and Gibbs free energy

In practice, situations are fairly frequently encountered where the temperature of the system is the same at the beginning as at the end of a process. Such processes may be regarded as isothermal from the viewpoint of their direction as well as their equilibrium. The chemical reactions in laboratory experiments are of this type, as are the life processes. Isothermal processes can also be studied on the basis of the entropy theorem as discussed in the previous section, if every part of the environment is regarded as belonging to the system, which therefore becomes isolated or at least adiabatic. This type of investigation is sometimes rather cumbersome and even unnecessary. In order to study isothermal processes, the use of the internal energy (enthalpy) and the entropy allows the introduction of state functions which give the direction and equilibrium of the processes directly. The state function which can be used in processes at constant volume (isochoric processes) is called the free energy, and the concept of Gibbs free energy is used to describe isobaric processes at constant pressure. The free energy function is also termed the *Helmholtz* function and the *Gibbs* free energy is sometimes called free enthalpy.

1. Free energy. Direction and equilibrium of isothermal and isochoric processes. The free energy is defined by the state function

$$F = U - TS \qquad [4.27a]$$

In isothermal processes, where T is constant

$$dF = dU - TdS \qquad [4.27b]$$

From experience it may be said that *isothermal processes proceed spontaneously in the direction of a decrease in the free energy. The end of the change of state, i.e. the equilibrium state, is characterized by the minimum free energy attainable in the given situation.* The decrease means a negative change, and the minimum attained refers to a zero change. These statements can be briefly expressed by the relation

$$dF \leqq 0 \qquad [4.28a]$$

dF has a simple physical meaning. To illustrate this, let us write the first law for a reversible process

$$dU = dQ_{rev} + dW_{rev} \qquad [4.28b]$$

Since the volume is constant in our case no volumetric work is done and dW_{rev} may denote some other, e.g. electric work. However, by definition $dQ_{rev} = TdS$, and thus [4.28b] can be rewritten to yield

$$dW_{rev} = dU - TdS \qquad [4.28c]$$

From a comparison of [4.27b] and [4.28c] we have

$$dF = dW_{rev} \qquad [4.28d]$$

which means that the change in the free energy in the case of isothermal–isochoric processes is equal to the work done in a reversible process, so that condition [4.28a] can be put into the form

$$dW_{rev} \leqq 0 \qquad [4.28e]$$

The work is less than zero if it is done by the system. *According to* [4.28e] *the isothermal–isochoric processes proceed spontaneously in the direction in which work is done by the system, or more exactly in the direction in which the system can do work.* This correction is necessary, since in reality the process may proceed without doing any work, only heat exchange occurring. However, in the determination of the direction of the spontaneous process information must be obtained about the possibilities. The maximum work which can be done by a system is that performed reversibly as expressed in [4.28e]. The system attains thermodynamic equilibrium, even if in principle no more work can be gained.

The correctness of [4.28a] can be proved by a simple example. Let us consider a perfect gas at temperature T enclosed in a vessel of volume $V = V_1 + V_2$, where the pressure in V_1 is p_1 and that in V_2 is p_2. The two volumes in the closed vessel are separated by a piston moving without friction, which allows a spontaneous pressure equalization. The walls of the vessel are made of some good heat-conducting material, so that the pressure is equalized at a constant temperature. We shall prove that in an isothermal–isochoric process the free energy of the total gas quantity decreases in accordance with [4.28], and the equilibrium is characterized by the minimum free energy. Let us apply the first law of thermodynamics to an elementary pressure equalization for both volume parts. In the case of a reversible process

$$dU_1 = TdS_1 - p_1 dV_1, \quad \text{and} \quad dU_2 = TdS_2 - p_2 dV_2$$

The change in free energy of the whole system, i.e. the quantity

$$dF = dU - TdS$$

is obtained by adding the two equations. This leads to the relation

$$dF = (p_2 - p_1)dV_1$$

The addition is carried out by using the fact that the total gas volume is constant, so that

$$dV = dV_1 + dV_2 = 0, \quad \text{and} \quad dV_2 = -dV_1$$

Further, it is taken into account that the internal energy and the entropy of the total system are obtained as the sums of the internal energies or entropies of the parts of the system (extensive quantities; cf. section 4.3.1), i.e. $dU = dU_1 + dU_2$ and $dS = dS_1 + dS_2$, respectively. From the resulting equation the following conclusions can be drawn:

(a) if $p_2 > p_1$, $dV_1 < 0$, and consequently $dF < 0$;
(b) if $p_2 < p_1$, $dV_1 > 0$, and consequently $dF < 0$;
(c) if $p_2 = p_1$, equilibrium exists, and $dF = 0$.

Thus, the conclusions fully prove [4.28a].

2. Gibbs free energy. Direction and equilibrium of isothermal–isobaric processes. The Gibbs free energy is defined by the state function

$$G = H - TS \qquad [4.29a]$$

which differs from the free energy only by substituting the internal energy by the enthalpy. The change in the Gibbs free energy in the case of isothermal processes can be described by

$$dG = dH - TdS \qquad [4.29b]$$

Isothermal and isobaric processes proceed spontaneously in the direction of a decrease in the Gibbs free energy. The equilibrium is characterized by the minimum value that can be reached in the given case. This can be written briefly as

$$dG \leqq 0 \qquad [4.30a]$$

In isothermal–isobaric processes, the change in the Gibbs free energy is equal to the reversibly performed work:

$$dG = dW_{rev} \qquad [4.30b]$$

Thus, instead of [4.30a] we may write

$$dW_{rev} \leqq 0 \qquad [4.30c]$$

which means that the isothermal–isobaric processes proceed spontaneously in a direction in which the system can do work along a reversible path.

The entropy, free energy, Gibbs free energy and chemical potential (to be introduced later; cf. section 4.5.1) in certain respects play the same role in Nature as the potential energy in pure mechanics or the electric potential in the field of electrical phenomena. The equilibrium state is characterized by a potential energy minimum, while the characteristics of the thermodynamic equilibrium are

— the entropy maximum in the adiabatic systems;
— the free energy minimum for isothermal–isochoric processes;
— the Gibbs free energy minimum for isothermal–isobaric processes.

We are thus justified in calling the above state functions *thermodynamic potentials.*

3. Determination of thermodynamic potentials. In this section only the Gibbs free energy will be dealt with, for in the applications mainly processes proceeding at constant pressure are encountered. However, the results also apply to the free energy. In the most frequent applications, in chemical reactions, the difference between the free energy and the Gibbs free energy cannot be neglected, especially for processes accompanied by gas formation or consumption. In these cases, it is advisable always to perform calculations with the Gibbs free energy, which otherwise practically agrees with the free energy.

The change in the Gibbs free energy can be *measured* directly only in cases, when a reversible change of state can be achieved to a good approximation and the work

done in this process is measurable. It holds generally that, in connection with chemical reactions, the change in the Gibbs free energy can be measured only in reactions functioning as reversibly working galvanic cells. *Calculation* of the Gibbs free energy is always possible if the enthalpy and entropy are known.

For instance, let us use the data in columns 3 and 4 of Table 4.5 and [4.29b], to calculate the change in the Gibbs free energy in the production of 1 mol water from 1 mol hydrogen and 1/2 mol oxygen at atmospheric pressure and 25 °C. In this case $\Delta H = 285.5$ kJ, $\Delta S = 69.9 - 130.6 - 102.6 = -163.3$ J/K, and $T\Delta S = -48.6$ kJ. Consequently, $\Delta G = -237.4$ kJ.[4] This value is given in column 5 of the Table, which also contains the Gibbs *free energy of formation* of other substances *related to 1 mol at 25 °C and 101 kPa.* These data are called *standard Gibbs free energies.* The Table shows that the Gibbs free energy is standardized similarly to the enthalpy, due to the fact that the absolute value of the Gibbs free energy is generally not known either, and only its changes can be calculated.

Table 4.5

Standard enthalpies (H°), standard entropies (S°) and standard Gibbs free energies (G°) of some substances

Element or compound	State	H° (kJ/mol)	S° (J/mol · K)	G° (kJ/mol)
H_2	g	0.0	130.6	0.0
O_2	g	0.0	205.2	0.0
C (graphite)	s	0.0	5.9	0.0.
H_2O	l	−286.0	69.9	−237.4
H_2O	g	−242.0	188.8	−228.6
CO_2	g	−394.0	214.0	−394.8
Acetic acid	l	−487.4	159.9	−392.7
Lactic acid	l	−677.0	192.2	−520.4
Ethyl alcohol	l	−278.0	160.8	−175.0
Glycerine	l	−666.6	208.1	−475.6
Glucose	s	−1280.1	212.4	−915.7

g = gas or vapour; l = liquid; s = solid

[4] The maximum work to be obtained in the formation of 1 mol water from 1 mol hydrogen and 1/2 mol oxygen can also be obtained from electrical data. Platinum electrodes surrounded by gaseous hydrogen and oxygen, respectively, are immersed in weakly acidified water. The pressure of the gas surrounding the electrodes is kept constant (e.g. at 101 kPa). The resulting system is a galvanic cell, whose electromotive force at 25 °C and 101 kPa is 1.23 V. The electric work is gained by the formation of water in the cell. By Faraday's law, an electric charge of 193,000 C passes through the system on the formation of 1 mol water. The maximum obtainable work is given by the product of the charge and the e.m.f. The result, as before is 237.4 kJ.

4. Bound energy. [4.29b] may also be written as

$$dH = dG + TdS$$

i.e. the change in Gibbs free energy is composed of two terms. The first gives the maximum work to be obtained from the enthalpy, and the second term provides information on the remainder, which cannot be used as work, but represents the *inevitable heat*. This latter term is called the *bound energy*. From Table 4.5, of the enthalpy liberated on the formation of 1 mol water from its elements a maximum of 237.4 kJ can be used as work, and at least 48.6 kJ is dissipated heat.

The statements concerning the direction of isothermal processes can be illustrated with the aid of the free energy or Gibbs free energy in the following way. Let us consider again the relation

$$dG = dH - TdS$$

and investigate separately the roles of the terms on the right-hand side. Two extreme situations may be conceived. The first involves processes in which the entropy does not change, while in the second case the enthalpy (internal energy) remains constant. The processes proceed spontaneously in the direction of enthalpy decrease in the first case, and in the direction of entropy increase in the second case. A decrease in enthalpy (internal energy) means energy liberation, which occurs if the attractive forces between the atoms or molecules become stronger in the process, which finally results in a more compact arrangement. An increase in entropy, on the other hand, is related to a decrease in the bonding between the particles, i.e. to structural loosening. Hence, two opposite effects exist, which together determine the direction of the process. For instance, the attractive forces predominate in condensation or in the synthesis of molecules, and the direction of the process is therefore determined by the decrease in enthalpy (internal energy). Conversely, scattering tendencies become dominant in evaporation, mixing, dissociation of molecules, etc., and in these cases the entropy increase will be the most important factor. It is generally true that the direction of the processes at sufficiently low temperature and high pressure is determined by the decrease in enthalpy (internal energy), whereas as the temperature is raised the entropy increase becomes increasingly more dominant.

4.5. Additions and applications

4.5.1. Gibbs free energy of mixtures. Chemical potential

The problems arising in practice are usually not associated with systems consisting of a single pure substance, but with mixtures (gas mixtures, solutions). The extra- and intracellular spaces are filled with mixtures, and metabolic processes also proceed in mixtures. Though the direction of the processes is unambiguously defined by the results derived in the previous section, their application still requires some consideration.

1. Ideal liquid mixtures. For simplicity, let us assume that the mixing occurs at 25 °C ($T \approx 298$ K) and a pressure of 101 kPa. The number of components is denoted

by n and the amount of the i-th component (in mol) by v_i. *The Gibbs free energy of the mixture is the sum of the Gibbs free energies of the components:*

$$G = \sum_{1}^{n} v_i \mu_i \qquad [4.31]$$

where μ_i denotes the Gibbs free energy of the i-th component in the case of the system examined relating to 1 mol of the component. Thus, μ_i is *partial molar Gibbs free energy*, also called the *chemical potential* of that component.

The task is to define μ_i. If the components were not in mixtures, but in their pure form (or in saturated solution), their Gibbs free energies relating to 1 mol at 25 °C and 101 kPa could be obtained from the tables. The same should be valid for the case, if the components were present in a concentration of 1 mol/l, for these data could also be found in tables. For instance the chemical potential of a glucose solution of unit molarity is −904 kJ/mol, that of lactic acid −530.1 kJ/mol. The value of μ_i in our case differs from the datum of tables, because the concentrations of the components in the mixture are generally different from those which the tables refer to. Let μ_i^0 denote, in the case of the i-th component, the chemical potential of the solution of unit molarity. For an ideal solution by using [4.24b] in the proper sense:

$$\mu_i - \mu_i^0 = RT \ln \frac{c_i}{c_i^0} \qquad [4.32a]$$

or

$$\mu_i = \mu_i^0 + RT \ln \frac{c_i}{c_i^0} \qquad [4.32b]$$

where $c_i^0 = 1$ mol/l, while c_i is the concentration of the i-th component in the solution.

μ_i^0 is referred to as *chemical normal potential* and its value at 25 °C is the *chemical standard potential*. The expression in [4.32b] with the concentrations is called *mixing term*. When formulating [4.32b] the concentration of the components was expressed in molarity (mol/l), but mole fractions could also have been used. The value of μ_i is independent of the concentrations used to characterize the composition of the mixture, but this does not hold separately for either the mixing term or for the chemical normal and standard potentials. The difference in the mixing term is a reasonable consequence of the fact that at a given composition the numerical values of the different concentrations are not equal. Further, the normalization and standardization refer in one case to a solution of unit molarity — as in [4.32b] too — and in the other to the pure state of the component, from which it follows that the normal and standard values are also different.

An additional remark: instead of [4.32b] one encounters very often the following form:

$$\mu_i = \mu_i^0 + RT \ln c_i \qquad [4.32c]$$

i.e. c_i^0 is omitted from the formula considering that its value is 1. From a dimensional point of view, of course, this form is not correct.

2. Real mixtures. Van't Hoff's law is only more or less valid for real mixtures, consequently these formulas of chemical potential hold only with better or worse approximation. However, this can be improved if the concentration is replaced by a new quantity which depends on the concentration so that the respective relations of ideal mixtures remain valid in their original form for real mixtures too. This quantity is the *activity* (a_i) in the case of liquid mixtures. The activity values can be determined and their relations with the concentrations are tabulated. Thus, the chemical potential of the *i*-th component for real mixtures, on the basis of [4.32c], can be written in the following form:

$$\mu_i = \mu_i^0 + RT \ln a_i \qquad [4.32d]$$

The chemical standard potentials are related to unit activity.

The findings for the Gibbs free energy also hold for the chemical potentials and in the case of mixtures they give information on the direction and the equilibrium of the changes in the individual components. If the chemical potential of one component is different at different points of the mixture, the respective component will migrate from a site of higher concentration to one of lower concentration. The overall mixture will be in equilibrium only if the chemical potential of each component is identical throughout the mixture.

From a study of the Gibbs free energy of mixtures, several practical results are obtained. For instance, the equilibrium constant of a chemical reaction or the equilibrium change due to a change in temperature or pressure can be calculated, and the chemical affinity too can be quantitatively characterized. Thermodynamic considerations lead to the derivation of a relation which permits a comparison of solution concentrations via measurement of the e.m.f. of a concentration cell. Similar relations can be derived between the potentials of redox systems and the concentrations of reduced and oxidized compounds. Some of the results will be dealt with in more detail below.

As concerns the metabolic reactions of the life processes, the change in the Gibbs free energy is itself of interest, because it gives information about the maximum mechanical, electric or other work which can be done at the cost of the energy liberated in the reactions, or which may be used to initiate other energy-requiring chemical processes. This is an important point, since the energy of the metabolic processes in the cells is usually expended to produce initially compounds (e.g. ATP) which have relatively large Gibbs free energies. The decomposition of these compounds at appropriate sites releases the energy necessary for the work required.

Let us calculate the Gibbs free energy change due to the decomposition of 1 mol glucose to 2 mol lactic acid at 25 °C and 101 kPa. The calculations are carried out for the case when the concentration of the mixture is 0.01 mol/l for glucose and 0.002 mol/l for lactic acid. These are the average concentrations in the living organism. The tabulated value of the chemical potential (standard potential) of a glucose solution of unit molarity is −904 kJ/mol, and that of the lactic acid solution is −530.1 kJ/mol. These values are changed by the mixing terms, which in the given case, according to [4.33b] are −11.3 kJ/mol for glucose and −15.5 kJ/mol for lactic acid. Thus, the chemical potentials are

−915.3 kJ/mol and −545.6 kJ/mol, respectively. Since 2 mol lactic acid is produced from 1 mol glucose, the Gibbs free energy change of interest is −175.9 kJ. The maximum work that can be obtained by the conversion of 1 mol glucose into lactic acid will therefore be 175.9 kJ. Lactic acid dissociates at 25.1 nmol/l H^+ concentration of the organism into hydrogen and lactate ions. When this process too is considered, the maximum work that can be obtained theoretically will be more: approximately 209.4 kJ. The organism uses this conversion in muscular activity, for instance. Measurements show that approximately 117.2 kJ work is gained, which corresponds to an efficiency of more than 50%. In reality the situation is even better, since the reaction takes place on the surface of the enzymes, where the concentrations differ from the data used above, i.e. from the mean concentration values. Near the surface of the enzymes the glucose concentration is obviously lower and the lactic acid concentration is higher than the mean value, since the glucose consumed cannot be replaced immediately and the lactic acid produced cannot move away at once from the site of its production. A further circumstance may be considered in connection with the efficiency. The work is actually measured on a group of several fibres and the individual fibres do not contract simultaneously. As a result, the measurements definitely yield values smaller than the actual ones.

It is worthwhile emphasizing in this context that the human organism is not a heat engine which transforms the heat produced into work; from a thermodynamic point of view, it should rather be regarded as a system which covers all the energy necessary for its functioning and activity not required as heat from the Gibbs free energy. With the aid of the enzymatic system, the processes occur nearly reversibly in the living organism. Consequently, from the aspect of the work performed, the organism is a nearly ideal thermodynamic system.

4.5.2. The quantitative description of chemical affinity

Let us consider a reversible chemical reaction at constant temperature and pressure. Reversible reactions are generally described by the stoichiometric equation

$$r_A A + r_B B + \ldots \rightleftharpoons r_K K + r_L L + \ldots \qquad [4.33a]$$

A, B, ... refer to the *initial substances*, and K, L, ... to the *products*. The quantities $r_A, r_B, \ldots r_K, r_L \ldots$ denote the stoichiometric coefficients in the studied processes. Instead of the above notation frequently the more concise symbolism

$$\sum r_A A \rightleftharpoons \sum r_Z Z \qquad [4.33b]$$

is used, where A stands for the initial substances and Z for the products.

In the course of the reaction the quantity of some substances decreases whereas that of others increases. These changes take place in ratios determined by the stoichiometric coefficients, which enables to describe the degree of the progress in any chemical reaction by a single quantity briefly called *reaction coordinate* (ξ). In order to determine the value of ξ one starts from the fact that while at the beginning of the process the product quantity is zero, it will be ν_K, ν_L, ... after some time.

Since

$$\nu_K : \nu_L : \ldots = r_K : r_L : \ldots \qquad [4.34a]$$

one may write

$$\nu_K = r_K\xi, \quad \nu_L = r_L\xi \tag{4.34b}$$

and for further changes one has

$$d\nu_K = r_K d\xi, \quad d\nu_L = r_L d\xi, \ldots \tag{4.34c}$$

ξ is a positive quantity which increases as the reaction advances. By similar reasoning the changes in the quantity of the initial substances are described by the equations

$$-d\nu_A = r_A d\xi; \quad -d\nu_B = r_B d\xi, \ldots \tag{4.34d}$$

The negative sign indicates the decrease of the quantity of the initial substances.

It is suitable to characterize the affinity among the substances participating in isothermal-isobaric reactions with a quantity which is positive in the direction of the spontaneous process and becomes the larger the further is the system from equilibrium, while in equilibrium this quantity is zero. Such quantity may be deduced from the change of the Gibbs free energy.

Recalling the reaction described by [4.33b] let us assume that it proceeds from left to right. The Gibbs free energy change of the total system will be

$$dG = \mu_A d\nu_A + \mu_B d\nu_B + \ldots + \mu_K d\nu_K + \mu_L d\nu_L + \ldots \tag{4.35a}$$

or in a more concise notation

$$dG = \sum \mu_A d\nu_A + \sum \mu_K d\nu_K \tag{4.35b}$$

where μ_A, μ_B ... and μ_K, μ_L ... denote the chemical potentials of the initial substances, as well as of the products in a given state of the system, while $d\nu_A$, $d\nu_B$ ... and $d\nu_K$, $d\nu_L$ ... indicate the changes in the quantity of the initial substances and the final products, respectively.

Equation [4.35b] may be rewritten in a better arranged form expressing the changes in the amount of substances with the reaction co-ordinates. By the use of relations [4.34c] and [4.34d] one has

$$d\nu_A = -r_A d\xi, \ldots \text{ and } d\nu_K = r_K d\xi, \ldots$$

consequently

$$dG = (\sum r_Z \mu_Z - \sum r_A \mu_A)\, d\xi \tag{4.35c}$$

Since for isothermal-isobaric processes

$$dG \leqq 0 \tag{4.36a}$$

(cf. section 4.4.5), taking into consideration [4.35c] we may write

$$(\sum r_Z \mu_Z - \sum r_A \mu_A)\, d\xi \leqq 0$$

and

$$\sum r_Z \mu_Z - \sum r_A \mu_A \leqq 0 \tag{4.36b–c}$$

respectively, where the inequality refers to spontaneous processes, and the equality indicates the equilibrium state.

Equations [4.36b–c] express the fact that the chemical reactions proceed in the direction in which the sum of the chemical potentials of the products weighted by their stoichiometric coefficients is smaller than that of the reacting substances. Equilibrium will be at equality.

The chemical affinity is characterized by the quantity

$$\mathbf{A} = -(\sum r_Z \mu_Z - \sum r_A \mu_A) \qquad [4.37a]$$

It clearly follows from the previous reasoning that A is positive and its value is the greater the further is the system from the equilibrium. In equilibrium $\mathbf{A} = 0$, consequently the above definition of affinity satisfies the previously formulated conditions.

In order to compare the affinity of the various reactions the affinity is normalized and/or standardized. The *normal affinity* (Å) related to the temperature T is characterized by the maximum work which would be done by the system at the given temperature and at the pressure of 101 kPa if the concentration (more exactly the activity) of the initial substances as well as the products were unit quantities. Without proof we give

$$\text{Å} = RT \ln K \qquad [4.37b]$$

where K denotes the equilibrium constant characteristic of the reaction (the definition has been given in section 4.5.3). The normal affinity at the temperature of 25 °C ($T \approx 298$ °K) is called *standard affinity*.

4.5.3. The law of mass action. Equilibrium constant

The determination of thermodynamic equilibrium for reversible chemical reactions is one of the fundamental and most important questions from the theoretical viewpoint as well as from practical aspects. The thermodynamic equilibrium is properly defined by the law of mass action, which is the result of the general thermodynamic equilibrium conditions (cf. section 4.4.5).

As has been already briefly pointed out, the chemical reactions are described by the stoichiometric equation

$$\Sigma r_A A = \Sigma r_Z Z$$

The r_A factors indicate the stoichiometric coefficients of the substances denoted by A, and the factors r_Z refer to the stoichiometric coefficients of the substances Z.

The direction of the reaction is determined by the activities (concentrations) which the components possess in the mixture. The activities are denoted by the symbols $a_A, a_B, \ldots, a_K, a_L \ldots$. The activities associated with the equilibrium state are denoted by a bar ($\bar{a}_A$, etc.). The relation existing between the equilibrium activities is called the law of mass action:

$$\frac{(\bar{a}_K)^{r_K}(\bar{a}_L)^{r_L}\cdots}{(\bar{a}_A)^{r_A}(\bar{a}_B)^{r_B}\cdots} = K \qquad [4.38a]$$

The quantity K, *the equilibrium constant*, is constant at given temperature and pressure and is characteristic of the given reaction.[5] If the activity of one of the components of the mixture is changed,

[5] For the temperature-dependence of the equilibrium constant, cf. section 1.4.5.

the others also change so that the value of K remains constant. The equilibrium constant can also be expressed in terms of chemical normal potentials of the individual components. With the usual notations, we have

$$\ln K = -\frac{\Sigma r_Z \mu_Z^0 - \Sigma r_A \mu_A^0}{RT} \qquad [4.38b]$$

The general derivation of the mass action law is omitted here. We shall restrict ourselves to a special case to illustrate the train of thought which leads to the law of mass action starting from the general conditions of thermodynamic equilibrium. Let us discuss, for example, the simple dissociation process:

$$C_{r_C} A_{r_A} \text{ (crystalline salt)} \rightleftharpoons r_C C + r_A A \text{ (dissolved salt)}$$

In the present case r_C denotes the stoichiometric number of cations C, and r_A that of anions A. Equilibrium exists if the Gibbs molar free energy of the salt in the solution is equal to the Gibbs molar free energy of the crystalline salt:

$$r_C(\mu_C^0 + RT \ln \bar{a}_C) + r_A(\mu_A^0 + RT \ln \bar{a}_A) = \mu_{CA}^0$$

The left-hand side refers to the salt in the solution, and the right-hand side to the crystalline salt. The first term of the left-hand side is associated with the cations, and the second one with the anions. μ_C^0 and μ_A^0 are the chemical normal potentials of the cations and anions in the solution while μ_{CA}^0 refers to the chemical normal potential of the crystalline salt. a_C and a_A are the *equilibrium* activities (concentrations) of the cations and the anions in the solution. After rearrangement, we have

$$r_C\mu_C^0 + r_A\mu_A^0 - \mu_{CA}^0 = RT \ln (\bar{a}_C)^{r_C}(\bar{a}_A)^{r_A}$$

Since the left-hand side is constant, the right-hand side must of necessity be constant too, from which it follows that

$$(\bar{a}_C)^{r_C}(\bar{a}_A)^{r_A} = K_{\text{diss}}$$

where the constant is now called the *solubility product*.

It can immediately be seen that the result is the special case of the law of mass action for unit denominator. In the present case the denominator is the activity characteristic of the crystalline salt, which can indeed be taken as unity.

In connection with the above example, the relation between the equilibrium constant and the chemical normal potential is obtained directly:

$$\ln K_{\text{diss}} = -\frac{r_C\mu_C^0 + r_A\mu_A^0 - \mu_{CA}^0}{RT}$$

Let us apply the above relation to the dissociation of water, i.e. to the process

$$H_2O \rightleftharpoons H^+ + OH^- \quad \text{or} \quad 2H_2O \rightleftharpoons H_3O^+ + OH^-$$

Calculation with the molar concentrations c_{H^+} and c_{OH^-} instead of the activities involves the relation

$$c_{H^+} \cdot c_{OH^-} = K_{\text{water}}$$

The value of K_{water} at 25 °C is 10^{-14} (mol/l).

4.5.4. Electrode potentials. Nernst's equation

The body fluids of the living organism contain various ions. The thermodynamic phenomena connected with these ions are of vital importance in the life processes. In the following section (cf. also sections 4.6.2 and 4.7.1) we shall deal with some basic relations necessary to obtain a deeper insight into the properties of electrolytes and other solutions containing charged particles.

1. Electrode potentials. If a metal (the electrode metal) is immersed into a solution of its ions, an electric potential difference results between the metal and the electrolyte. Its production can be interpreted in the following way. When the metal is immersed in the electrolyte, depending upon the concentration (activity) positive metal ions either pass from the metal into the solution, or vice versa. In the former case the metal will become negatively charged, while in the latter case it will be positively charged with respect to the solution. The potential difference between the electrode and the solution will in both cases increase to a certain extent and within a fairly short time *dynamic equilibrium* will be established at the boundary surface between the metal and the solution. Here too ions move from one phase into the other, but in a given time the voltage produced will cause the same number of ions to move in the opposite direction. The potential difference associated with the equilibrium state is the *electrode potential.*

Similar processes take place at both electrodes of galvanic cells, and the e.m.f. of the cell results from the *algebraic difference* of the two electrode potentials.

The electrode potential ε can be calculated from thermodynamic considerations (see below). The relation

$$\varepsilon = \varepsilon^0 + \frac{RT}{Fz} \ln a \qquad [4.39a]$$

is obtained, where R is the universal gas constant, T is the temperature, F is Faraday's constant, z is the valence of the electrode metal ions, and a denotes the activity of the metal ions in the solution. ε^0 is the electrode potential of a solution of unit activity; it is called the *normal electrode potential* at a pressure of 101 kPa. The normal potential at 25 °C is the *standard electrode potential* of the respective electrode.[6] By convention, the values ε and ε^0 are positive if the electrode metal has a positive potential with respect to the electrolyte, while in the opposite case ε and ε^0 are negative.

Electrode potentials are measured in the following way. A galvanic cell is made, one electrode of which is the investigated metal together with the electrolyte in contact with the metal,[7] while the other electrode is the reference electrode, usually a standard hydrogen electrode (see below). The electrode potential of the studied electrode can be characterized by the e.m.f. of this cell. The normal potential of the standard hydrogen electrode used for comparison is taken arbitrarily as zero at the temperature of measurement. The tabulated data are usually the potentials of electrodes relative to the standard hydrogen electrode at 25 °C.

The thermodynamic considerations concern the case when the electrode metal dissolves on being immersed in the solution. Thermodynamic equilibrium between the metal and the solution is reached when the work associated with the transition of the ions is zero. In the present case this work consists

[6] Many authors use the expression electrochemical normal potential and electrochemical standard potential instead of normal electrode potential and standard electrode potential, respectively. The expression electrochemical potential is used in this book, too, but in another sense (cf. section 4.6.2).

[7] In the present case the name electrode may mean not only the metal conductor immersed in the electrolyte, but (as frequently used in electrochemistry) the *system* consisting of the respective element and the solution containing its ions.

of two parts: the Gibbs free energy change (W_1) accompanying the dissolution of the metal, and the electric work (W_2) due to the potential difference between the metal and the solution. Both components are related to 1 mol ions. With the usual notations, W_1 is given by

$$W_1 = \mu_{ion} - G_{metal} \quad \text{or} \quad W_1 = \mu^0_{ion} + RT \ln a_{ion} - G_{metal}$$

The electric work is

$$W_2 = zF(\varphi_{solution} - \varphi_{metal})$$

The potential difference in brackets is written as a difference with regard to the further discussion (cf. point 2). At equilibrium, $W_1 + W_2 = 0$; i.e.

$$\mu^0_{ion} + RT \ln a_{ion} - G_{metal} + zF(\varphi_{solution} - \varphi_{metal}) = 0$$

from which the electrode potential of interest is

$$\varphi_{metal} - \varphi_{solution} = \frac{\mu^0_{ion} - G_{metal}}{zF} + \frac{RT}{zF} \ln a_{ion}$$

or with a simplified notation

$$\varepsilon = \varepsilon^0 + \frac{RT}{zF} \ln a$$

Thus, [4.39a] has been proved.

2. The Nernst equation. Galvanic cells whose electrodes are identical, and in which only the concentrations (activities) of the electrolyte solutions around the two electrodes are different, are called *concentration cells*. The production of e.m.f. can be interpreted according to the scheme outlined in point 1, since [4.39a] shows that the electrode potential depends upon the concentration of the solution. Consequently, different electrode potentials are created on the two electrodes of the cell. The e.m.f. (disregarding the liquid potential) is given by their algebraic difference, which means that the e.m.f. of the concentration cell is

$$E = \varepsilon_1 - \varepsilon_2 = \frac{RT}{zF} \ln \frac{a_1}{a_2} \qquad [4.39b]$$

where the subscripts 1 and 2 are used to distinguish the two electrodes. [4.39a] or [4.39b] is called the *Nernst equation.*

[4.39b] provides an easy method of determining the ion concentration: if the e.m.f. is measured, the concentration of one solution can be calculated if that of the other is known. A particularly frequent task in practice is the determination of the hydrogen ion concentration of a solution. For this purpose hydrogen electrodes are used, which belong in the group of gas electrodes. A gas electrode too contains a metal, but it is always surrounded by the corresponding gas, and the metal is only the gas carrier. For the hydrogen electrode the carrier electrode is platinum and the whole system is immersed in a solution containing hydrogen ions. Two electrodes are always required to carry out the measurements: one is immersed in the solution to be studied, and the other in the solution used for comparison. In the case of hydrogen ions a molar solution (strictly speaking a solution of unit activity) is used, and this system is then the *standard hydrogen electrode.* Since the hydrogen electrode is sensitive only to the hydrogen ion concentration, this procedure can be applied to solutions (e.g. blood) which contain many other components, so that any other method of determination would be complicated and less exact. With appropriate electrodes, the concentrations of other ions can be determined in a similar way.

4.5.5. Some remarks

As has been mentioned previously, the first law of thermodynamics given in the form of equation [4.14] may be thought of as a general law. However, a more specific formulation can be presented. This will be demonstrated in a case when *thermal, mechanical* and *chemical* interactions exist in isothermal and isobaric conditions of a chemical system. The changes of the internal energy resulting from these interactions may be written for the quasi-static case in the form

$$dQ = T\,ds;\ dW_{\text{mech}} = -p\,dV;\quad dW_{\text{chem}} = \sum_{1}^{n} \mu_i\,dv_i \qquad [4.40a]$$

consequently

$$dU = T\,ds - p\,dV + \sum_{1}^{n} \mu_i\,dv_i \qquad [4.40b]$$

Note that each energy or work term on the right side may be constructed as the product of the intensive and extensive quantity (more exactly of the change of the extensive quantity) corresponding to the relevant interaction. This statement is also valid for other interactions not considered here. Accordingly the quantities related to these interactions, including also the electrostatic interactions, are collected in Table 4.6.

Table 4.6

Quantities characterizing energetic interactions

Interaction	Characteristic quantity		Work or energy
	extensive	intensive	
Mechanical	volume (V)	pressure (p)	volumetric work ($-pdV$)
Electrostatic	electric charge (q)	electric potential (φ)	electric work (φdq)
Chemical	amount of component (v_i)	chemical potential of component (μ_i)	work required for transport of molecules ($\mu_i dv_i$)
Thermal	entropy (S)	temperature (T)	heat ($T\,dS$)

Thereupon the first law takes the simple form

$$dU = \sum_{1}^{s} y_i\,dx_i \qquad [4.40c]$$

where x_i and y_i are the intensive and the extensive quantities characterizing the i-th interaction, and s denotes the number of interactions.

4.6. Non-equilibrium processes

In our discussion of thermodynamic processes and the determination of their direction, we have been engaged mainly with the study of equilibrium states. In the following treatment, where the results of equilibrium thermodynamics are applied, we return to non-equilibrium processes, such as diffusion, the flow of heat and electric charge, etc. All these phenomena will be reviewed from a uniform aspect.

4.6.1. Onsager's linear relations

Any isolated system is in equilibrium only if the intensive quantities are the same at every point of the system. This statement is frequently referred to as the *zero-th law of thermodynamics*. If this condition is not fulfilled *transport processes* (flows) are produced which lead to the equalization of the differences among the various intensive quantities. In all of these processes some extensive quantity flows, as for instance the volume-flow leading to the equalization of pressure differences, the flow of charges (the electric current), the material flow resulting from the differences of the chemical potentials in chemical reactions as well as in dissolution and precipitation processes, etc.

The transport is characterized by the *flux (current density)* of the flowing material. The flux is given by the quantity passing through unit cross-section in unit time. The transport is due to the inhomogeneity in the spatial distribution of the intensive quantities, which is characterized by the *gradient* of these quantities. For simplicity we discuss only cases where a single quantity participates in the process, and its value changes only in one dimension, the x coordinate, the positive X axis pointing in the direction of the transport. Let us denote the change in the intensive quantity in question over the length dx by dy. The gradient is then defined as dy/dx. The gradient of the intensive quantities plays the same role in thermodynamics as the force in mechanics. For this reason the gradient of the intensive quantities, or more exactly its negative value, is called the *generalized* or *thermodynamic force*.

A comparison of the equations describing the various transport processes (Ohm's law, Fick's first law, the Hagen–Poiseuille law, etc.) results in the following general statement: *the flux (J) is proportional to the corresponding thermodynamic force (X)*, i.e.

$$J \sim X, \quad \text{or} \quad J = LX \qquad [4.41]$$

where the coefficient L is called the *phenomenological coefficient*. Of course, L does not contain the gradient explicitly, though it may contain several quantities associated with the transport investigated, among them some which appear in the gradient. The phenomenological coefficient cannot be derived thermodynamically; in individual cases it can be obtained empirically or by reasoning. This also follows from

the equations describing the transport processes. The above statement will be referred to in the forthcoming discussions as *Onsager's linear law*.[8]

Let us study the *diffusion* more closely. In this case the thermodynamic force is the chemical potential gradient of the solute, and we have

$$X = -\frac{d\mu}{dx} \qquad [4.42a]$$

where $d\mu$ is the chemical potential change along the length dx (Fig. 4.13*a*). In the present case the flux is

$$J = \frac{dv}{dt}\frac{1}{q} \qquad [4.42b]$$

where dv denotes the amount of substance transported across the surface q in time dt (Fig. 4.13*b*). From [4.41]

$$\frac{dv}{dt}\frac{1}{q} \sim -\frac{d\mu}{dx} \qquad [4.42c]$$

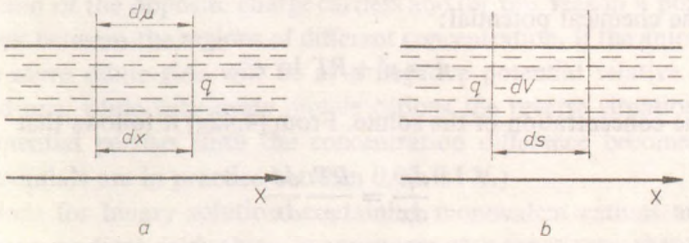

Fig. 4.13. Diagram for the interpretation of diffusion
a: notations associated with thermodynamic force; *b:* notations associated with flux.
The X axis simultaneously denotes the direction of the thermodynamic force and the flux

In the following we shall prove that [4.42c] corresponds to Fick's first law, and additional information will be obtained on the diffusion coefficient.

Let c denote the concentration of the solute, and dV the volume of solution containing the amount of solute dv. It follows from the concept of the concentration of substance that $dv = cdV$.

It is also taken into consideration that $dV = qds$, where ds denotes the distance moved by the diffusing molecules in time dt in the direction of diffusion. ds/dt gives the mean diffusion velocity of the molecules, which will be denoted by v. The flux can then be expressed as

$$J = cv \qquad [4.42d]$$

[8] In most thermodynamic systems several thermodynamic forces act and various quantities flow. The linear relations between these quantities are called Onsager's linear equations (cf. section 4.7.3). [4.41] describes the special case when only one thermodynamic force acts, resulting in the transport of only one extensive quantity.

This relation can be transformed if the diffusion of the molecules is considered as frictional motion, and use is made of the empirical relation (cf. section 4.1.3) that the velocity of a moving particle is proportional to the driving force. The driving force in the present case is the thermodynamic force given in [4.42a], and hence

$$v = -u\frac{1}{N_A}\frac{d\mu}{dx} \qquad [4.42e]$$

where u denotes the mobility of the solute molecules. The division by the *Loschmidt constant* (Avogadro's constant, N_A) is explained by the requirement that in [4.42e] we have to calculate with the force acting on one single molecule, whereas $d\mu/dx$ represents the force acting on one mol. With the use of [4.42e], [4.42d] can be rewritten as

$$J = -uc\frac{1}{N_A}\frac{d\mu}{dx} \qquad [4.42f]$$

which is a more developed form of [4.42c], since it is an equality instead of a proportionality. In the next step we transform the gradient $d\mu/dx$ by making use of relation [4.32c] for the chemical potential:

$$\mu = \mu^0 + RT \ln c \qquad [4.42g]$$

where c is the concentration of the solute. From [4.42g] it follows that

$$\frac{d\mu}{dx} = \frac{RT}{c}\frac{dc}{dx} \qquad [4.42h]$$

Consequently

$$J = -ukT\frac{dc}{dx} \qquad [4.42i]$$

where $k = R/N_A$ is the Boltzmann constant.

It is clear that [4.42i] is identical with Fick's first law, and the diffusion coefficient is given by the equation

$$D = ukT \qquad [4.43a]$$

For spherical particles we have from [4.4b] $u = 1/6\pi\eta r$, and thus

$$D = \frac{kT}{6\pi\eta r} \qquad [4.43b]$$

In this way, Einstein's relation defining the diffusion coefficient has been proved.

In connection with [4.41], we also refer to Ohm's law on electric current. This states that the electric current density is proportional to the electric potential gradient, the proportionality factor being the specific conductivity. In this concept Ohm's law not only represents the proportionality between the thermodynamic force and the flux, but also gives the phenomenological coefficient.

Besides strict transport processes, chemical processes too can be treated in a similar way if a relation similar to [4.41] can be found. Instead of the flux, in these cases the calculations involve the rate of formation or depletion of one of the participating components, and the role of the thermodynamic force inducing the process is taken over by the affinity. Consequently, the following statement corresponds to [4.41]: *the rate of formation (or depletion) is proportional to the chemical affinity.*

4.6.2. Diffusion of electrolytes. Diffusion potential

The bringing-together of electrolytes with different concentrations (activities) may lead to the development of an electric potential gradient, the *diffusion potential* (or more exactly the diffusion voltage). This process may be visualized in the following way. Diffusion is always directed from higher to lower concentrations. If the anion and cation mobilities are the same, they diffuse together (quite randomly) and no voltage develops. However, if their mobilities are different, the more mobile ions lead the way and the less mobile ions lag behind. As a result, some order develops in the distribution of the opposite charge carriers and for this reason a potential difference develops between the regions of different concentration. If the anion is the more mobile, the more dilute side will be at a negative potential relative to the more concentrated one, while with more mobile cations the reverse situation arises. The diffusion potential persists until the concentration difference becomes zero. (The diffusion potentials are in practice between 0.01–0.1 V.)

Calculations for binary solutions containing monovalent cations and anions in a concentration gradient dc/dx show, in agreement with experience, that the following relation holds for the potential gradient

$$\frac{d\varphi}{dx} = -\frac{RT}{F}\frac{u_C - u_A}{u_C + u_A}\frac{1}{c}\frac{dc}{dx} \qquad [4.44a]$$

where c is the concentration of the electrolyte in the volume in question, R is the universal gas constant and F is Faraday's constant. The subscript C means the cation, and A the anion.

Integration of [4.44a] yields

$$\varphi_2 - \varphi_1 = \frac{RT}{F}\frac{u_C - u_A}{u_C + u_A}\ln\frac{c_1}{c_2} \qquad [4.44b]$$

[4.44b] defines the potential difference that develops in the contact layer of solutions of concentrations c_1 and c_2.

It is clear from the above equations and also from the qualitative description that the larger the mobility difference between the anions and cations and the concentration ratio of the solutions in contact, the larger is the diffusion potential. If no concentration gradient exists or the mobilities are the same, the diffusion potential is zero.

Table 4.7

Mobilities of some ions (in relative units) in aqueous solution at infinite dilution at 25 °C

Ion	Mobility	Ion	Mobility	Ion	Mobility
H^+	349.8	Mg^{2+}	53.0	OH^-	198.6
Li^+	38.7	Ca^{2+}	59.5	F^-	55.4
Na^+	50.1	Sr^{2+}	59.4	Cl^-	76.4
K^+	73.5	Ba^{2+}	63.6	Br^-	78.1
Rb^+	77.8			I^-	76.8
Cs^+	77.2			$CH_3CO_2^-$	40.9
				SO_4^{2-}	80.0

The mobilities of the individual ions can be determined via their electric conductivities, since a linear relation exists between the two quantities. Table 4.7 lists a few data. The diameter of the alkali metal ions increases on proceeding from Li^+ to Cs^+; their mobilities change in the same sense. At first sight it would appear that this observation contradicts the idea that the diffusion may be compared to the motion of spherical particles in a viscous medium, since the mobility and the particle diameter are inversely proportional to each other in the case of frictional motion. However, the apparent contradiction is explained by taking into consideration that the ions are enveloped in hydrate shells and, as concerns the mobility, not the diameter of the ion but that of the hydrate shell moving together with the ion is decisive. The diameter of the hydrate shell, however, decreases from Li^+ to Cs^+. The mobilities of the H^+ or H_3O^+ ion and the OH^- ion are considerably larger than would be expected from the diameters of their respective hydrate shells. This irregular behaviour is explained by proton exchange. In the case of the H_3O^+ ion this means that the H_3O^+ transfers a H^+ ion to a neighbouring water molecule, which is equivalent to the propagation of the H_3O^+ ion. The OH^- ion, on the other hand, migrates by abstracting a proton from a neighbouring water molecule. It should be observed that the mobilities of the K^+ and Cl^- ions are practically equal. This means that for KCl solutions the diffusion potential can be taken as zero. For this reason, a concentrated KCl solution is used as liquid junction when a diffusion potential is to be avoided (cf. the measurement of the resting potential in section 6.1).

The relation describing the diffusion potential can be obtained in the following way. For the flux of any ion in an electrolyte a relation similar to [4.42f] holds

$$J = -uc\frac{1}{N_A}\frac{d\mu^e}{dx} \qquad [4.45a]$$

However, in the present case the driving force is not only the chemical potential gradient; the force due to the electric potential gradient must also be considered. Their resultant is called the *electrochemical potential gradient*, which is denoted in [4.45a] by $d\mu^e/dx$. Consequently

$$\frac{d\mu^e}{dx} = \frac{d\mu}{dx} + zF\frac{d\varphi}{dx} \qquad [4.45b]$$

Only the product zF in the second term on the right-hand side requires explanation. In [4.45b] the thermodynamic forces refer to 1 mol amount of substance. However, $d\varphi/dx$ gives only the force acting on unit charge. The electric force on 1 mol of ions

will be obtained if $d\varphi/dx$ is multiplied by the charge of 1 mol ion, i.e. by the quantity zF. The electrochemical potential is clearly given by the expression

$$\mu^e = \mu + zF\varphi \qquad [4.45c]$$

From [4.42h], [4.45b] can be rewritten as

$$\frac{d\mu^e}{dx} = \frac{RT}{c}\frac{dc}{dx} + zF\frac{d\varphi}{dx} \qquad [4.45d]$$

and we have

$$J = -ukT\left(\frac{dc}{dx} + \frac{zcF}{RT}\frac{d\varphi}{dx}\right) \qquad [4.45e]$$

For simplicity, we calculate the diffusion potential only for binary solutions containing monovalent cations and anions, that is we shall prove only [4.44a]. For our purpose we write the flux of the monovalent cations and anions with the aid of [4.45e]:

$$J_C = -u_C kT\left(\frac{dc}{dx} + \frac{cF}{RT}\frac{d\varphi}{dx}\right) \quad \text{and} \quad J_A = -u_A kT\left(\frac{dc}{dx} - \frac{cF}{RT}\frac{d\varphi}{dx}\right) \qquad [4.46a]$$

Since the diffusion of the ions in itself (i.e. without an external electric field) does not produce an electric current,

$$J_C - J_A = 0 \qquad [4.46b]$$

and

$$u_C\frac{dc}{dx} + \frac{u_C cF}{RT}\frac{d\varphi}{dx} - u_A\frac{dc}{dx} + \frac{u_A cF}{RT}\frac{d\varphi}{dx} = 0 \qquad [4.46c]$$

If this is rearranged to express the gradient $d\varphi/dx$, [4.44a] is obtained directly.

4.7. Transport across membranes

In the life processes, the nutrient molecules, the intermediate and final metabolic products, etc. in most cases do not diffuse in a continuous medium. The membranes covering the cells and certain cell components are permeable to various extents for different substances and participate actively in the processes; they therefore exert a profound influence on the transport processes, and hence on the state of the intra- and extracellular space. In the following section we shall discuss the characteristics of transport processes across the membranes.

4.7.1. Membrane equilibrium and membrane potentials

If a membrane is perfectly permeable for both the solvent and the solutes, at equilibrium the concentrations (activities) on the two sides of the membrane will be the same. From a practical point of view, however, the processes of interest are those

in which the membrane is permeable for only some components, and is impermeable for the others. The osmotic processes already dealt with are of this type, as are the phenomena in solutions containing ions and other charged particles.

1. A simple case of membrane equilibrium. Let us consider the simple case when the same electrolyte is situated on both sides (I and II) of the membrane, but in different concentrations, and the membrane is permeable only for the cation of valence z_C. Thus, only the cations can migrate from side I (higher concentration) to side II, the anion migration being stopped by the membrane. As a result an *electric double layer* is formed on the membrane: one layer consists of the anions stopped on side I, while on side II there is a layer of cations attracted by the anions. The concentration difference "drives" the cations, whereas the electric field of the double layer "pulls them back". At equilibrium the potential difference between the two sides of the membrane ($\varphi^{II}-\varphi^{I}$) and the cation concentrations on the two sides (c_C^{I} and c_C^{II}) at a given temperature T are related in the following way:

$$\varphi^{II}-\varphi^{I}=\frac{RT}{z_C F}\ln\frac{c_C^{I}}{c_C^{II}} \qquad [4.47a]$$

where R is the universal gas constant and F is the Faraday constant.

[4.47a] can be derived in various ways. One is to write the electrochemical potentials of the cation for the two sides. At equilibrium the two electrochemical potentials are equal and [4.47a] follows dis rectly from this. [4.47a] can also be obtained by considering the diffusion potentials, i.e. [4.44b]. In the present example the membrane obstructs the diffusion of the anion, so that $u_A=0$. In this special case [4.44b] gives [4.47a].

2. Donnan equilibrium. Donnan voltage. Figure 4.14 depicts a somewhat more complicated case. The membrane is fully permeable for the monovalent ions C^+ and A^- of the electrolyte CA but it is impermeable for the similarly monovalent molecular ions R^- on side II. The freely diffusing C^+ and A^- ions are said to be mobile, while the R^- ions stopped on side II of the membrane are immobile. Because of the presence of these latter ions, neither of the mobile ions is evenly distributed on the two sides of the membrane, and a special equilibrium, the *Donnan equilibrium,* is formed. (The expressions in brackets in Fig. 4.14 denote the equilibrium concentrations.) The development of the equilibrium along the membrane is accompanied by the formation of an electric double layer, the equilibrium voltage of which is called the *Donnan voltage.*

The Donnan equilibrium follows from the general conditions of thermodynamic equilibrium. In the case under discussion, in agreement with experience, the following relation holds between the equilibrium concentrations:

$$c_C^{I}c_A^{I}=c_C^{II}c_A^{II} \quad \text{and} \quad \frac{c_C^{I}}{c_C^{II}}=\frac{c_A^{II}}{c_A^{I}}=r \qquad [4.47b]$$

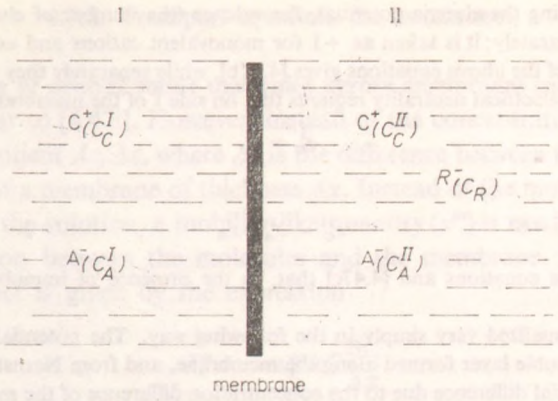

Fig. 4.14. Diagram relating to development of Donnan equilibrium

According to the left-hand equation, the products of the mobile monovalent ion concentrations are equal on the two sides of the membrane. The equation on the right expresses that the ratio of the different cation or anion concentrations on the two sides of the membrane gives the same number r. Due to the presence of the immobile ions, this number is not unity; it is called the *Donnan ratio*. The Donnan voltage is given by the following equation

$$\varphi^{II} - \varphi^{I} = \frac{RT}{F} \ln \frac{c_C^I}{c_C^{II}} = \frac{RT}{F} \ln \frac{c_A^{II}}{c_A^I} = \frac{RT}{F} \ln r \qquad [4.47c]$$

If the system contains several types of mobile ions, including *multivalent* ones, power relations hold instead of the above simple concentration ratios, and in this case r denotes their common value. The exponents are different for the individual ions, and are the reciprocals of their valences. Let us denote the valence of the i-th ion by z_i, and the equilibrium concentrations by c_i^I and c_i^{II}, respectively; in this case

$$r = \left(\frac{c_i^I}{c_i^{II}} \right)^{1/z} \qquad [4.47d]$$

The expression for the Donnan potential is formally the same in the general case as in the simple example discussed here, except that r must be substituted by [4.47d]. z_i is a positive integer for the cations and a negative integer for the anions.

As already mentioned, the relations for Donnan equilibrium follow from the general conditions of thermodynamic equilibrium. The calculations are carried out in connection with the example outlined in Fig. 4.14. The electrochemical potentials at equilibrium are equal on the two sides of the membrane. The equation must hold separately for the cations C^+ and the anions A^-, and consequently, with the usual notations, we may write

$$\mu_C^0 + RT \ln c_C^I + F\varphi^I = \mu_C^0 + RT \ln c_C^{II} + F\varphi^{II}$$
$$\mu_A^0 + RT \ln c_A^I - F\varphi^I = \mu_A^0 + RT \ln c_A^{II} - F\varphi^{II}$$

In the terms containing the electric potential, the valence (the number of charges on the ion) has not been denoted separately; it is taken as $+1$ for monovalent cations and as -1 for monovalent anions. Summation of the above equations gives [4.47b], while separately they yield [4.47c].

The condition of electrical neutrality requires that on side I of the membrane

$$c_C^{\mathrm{I}} = c_A^{\mathrm{I}}$$

and on side II

$$c_C^{\mathrm{II}} = c_A^{\mathrm{II}} + c_R$$

It follows from these equations and [4.47c] that, in the presence of immobile anions, $r<1$ and $c_C^{\mathrm{II}} > c_A^{\mathrm{II}} < c_A^{\mathrm{I}}$.

[4.47c] can be visualized very simply in the following way. The potential difference $\varphi^{\mathrm{II}} - \varphi^{\mathrm{I}}$ is the voltage of the double layer formed along the membrane, and from Nernst's equation the other terms give the potential difference due to the concentration difference of the mobile ions on the two sides of the membrane. Expressed in a different way: the Donnan membrane potential figures on the left-hand side, and the other expressions give the voltage measured if the solutions with appropriate electrodes constitute a concentration cell. At equilibrium the two voltages are the same in magnitude, but opposite in sign.

In a given case the measurement of both voltages is possible and the results prove the above statement. If electrodes of some mobile ion are immersed in the solutions on the two sides of the membrane, no potential difference can be measured between the two electrodes, because the voltage of the concentration cell is neutralized by the Donnan voltage of equal magnitude but opposite sign. However, if the equilibrium concentration conditions are not disturbed, but the system is transformed so that the solutions are connected by concentrated KCl solution instead of the membrane, the membrane potential is excluded, and only the concentration voltage of those ions whose electrodes are immersed in the solution will be measured (Fig. 4.15*a*; the intermediate KCl solution also decreases the diffusion potential). On the other hand, if the membrane is left in place and the solutions are connected through concentrated KCl solution to *identical* electrodes (in order to increase the accuracy to two non-polarizing, e.g. calomel electrodes), the system will be insensitive to the concentration voltage and only the Donnan voltage will be measured (Fig. 4.15*b*).

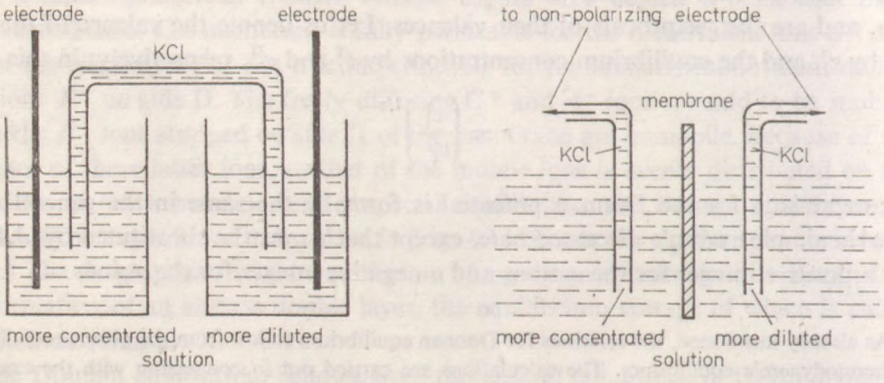

Fig. 4.15. Outlines of measurement of concentration potential (*a*) and Donnan potential (*b*)

4.7.2. Transport equations for membranes

1. The diffusion of neutral solute molecules across membranes can be described by an equation similar to [4.42i]. However, instead of the concentration gradient dc/dx we employ the quotient $\Delta c/\Delta x$, where Δc is the difference between the concentrations on the two sides of a membrane of thickness Δx. Instead of the mobility of the molecules diffusing in the solution, a mobility-like quantity (u^m) is used, which characterizes the interaction between the molecules and the membrane. Thus, the flux of the i-th component is given by the expression

$$J_i^m = -u_i^m kT \frac{\Delta c_i}{\Delta x} \qquad [4.48a]$$

(The superscript m refers to the presence of the membrane.) Quite frequently, neither u_i^m nor Δx is known exactly, and for this reason they are combined into one quantity which also includes Boltzmann's constant (k) and the temperature (T). The resulting quantity is called the *permeability constant* for the i-th component and is denoted by p_i. Thus

$$J_i^m = -p_i \Delta c_i \qquad [4.48b]$$

p_i is the flux measured in the case of unit concentration difference.

2. The transport of ions (and other electrically charged particles) in solution may be induced not only by a concentration gradient, but also by an electric potential gradient. In the case of biological membranes, both factors act simultaneously, for it is found by experience that the concentrations are different on the two sides of the membranes and an electric potential difference too is measured between the two sides. The electric potential difference (otherwise the electric double layer) is a result of the fact that the permeability of the membrane is different for the different ions in its environment. Consequently, both a concentration gradient and an electric potential gradient exist inside the membrane. In this case the flux of the k-th ion in the membrane is described by a relation differing from [4.45c] only by the constant $z_k e$ (the charge of the k-th ion):

$$J_k = -u_k z_k e k T \left(\frac{dc_k}{dx} + \frac{F z_k c_k}{RT} \frac{d\varphi}{dx} \right) \qquad [4.49]$$

The difference is due to the fact that [4.45e] holds for the material flow of the ions, whereas [4.49] refers to the electric charge flow of the ions.

From [4.49] it is possible to derive a relation for the *membrane potential*. We do not carry out the calculations, but discuss only the conditions applied and the results obtained.

For biological membranes it may be assumed that the electric potential gradient is constant across the membrane, so that $d\varphi/dx$ may be replaced by the expression

$(\varphi_i - \varphi_e)/\delta$, where φ_i and φ_e are the electric potentials on the internal and external sides of the membrane, and δ denotes the membrane thickness. Further, it is a well-founded assumption that the ion transport in the membrane is a *stationary* process, i.e. J_k is independent of time. This condition means that J_k is independent of position too: neither charge accumulation, nor charge depletion occur between two arbitrarily selected points of the membrane. Moreover, in full agreement with experience, it may be stated that a charge transport across the membrane produces an electric current only if the system is placed in an external electric field. Under normal circumstances the processes in biological systems proceed without an external electric field, and hence the electric flux flowing through the membranes is zero.

On the above basis, the following expression can be derived for the membrane potential

$$\varphi_i - \varphi_e = \frac{RT}{F} \ln \frac{\sum_{k=1}^{m} p_k^+ c_{ke}^+ + \sum_{k=1}^{n} p_k^- c_{ki}^-}{\sum_{k=1}^{m} p_k^+ c_{ki}^+ + \sum_{k=1}^{n} p_k^- c_{ke}^-} \qquad [4.50]$$

where only the n species of monovalent anions and the m species of similarly monovalent cations have been taken into account, since mainly these participate in the ion transport in biological membranes. The plus and minus signs refer to the positive and negative ions, c_{ki} and c_{ke} denote the ion concentrations of the solution on the internal and external sides of the membrane, and p_k is the permeability constant of the ions relative to the potassium ion.

3. The transport of water. Water is a basic component of the living cell, and consequently a discussion of some of the characteristic features of its transport is justified. Most biological membranes are permeable to water, but they display differences in their tolerance of hydrostatic pressure differences. The glomerular membranes are relatively rigid and are thus able to maintain relatively large pressure differences. Red blood cells are less rigid, and the membranes of amoebae, for example, are even less rigid. In plant cells the rigid cellulose matrices enable the thin membranes to endure relatively large pressure differences.

The flux J_{water}^m across a membrane is determined by two types of pressure differences: the hydrostatic pressure difference (Δp_{hst}) between the two sides of the membrane, and the osmotic pressure difference (Δp_{osm}) due to the concentration differences. The flux of water is proportional to the algebraic sum of these quantities:

$$J_{\text{water}}^m = -p_{\text{water}}(\Delta p_{\text{hst}} - \Delta p_{\text{osm}}) \qquad [4.51]$$

The proportionality factor p_{water}, the *hydrodynamic permeability coefficient*, depends upon the mobility of water in the membrane and the membrane thickness. In order to explain the signs, it is enough to remember that the direction of water transport is determined by the direction of the hydrostatic pressure decrease and by that of the osmotic pressure increase.[9] It depends upon the relative magnitudes and signs of Δp_{hst} and Δp_{osm} whether in a given case the water flows from the more dilute to the more concentrated solution or in the opposite direction. With a sufficiently large

[9] [4.51] is a consequence of Onsager's law; its derivation is omitted, since the result is simple and illustrative.

hydrostatic pressure it can be attained that the water is forced from the solution of higher concentration into the solution of lower concentration. This phenomenon is called *ultrafiltration*, which plays an important role in biological material transport and is also utilized in practice. As an example, [4.51] explains the fact that liquid efflux occurs at the arterial end of capillaries, whereas influx takes place at the venous end.

According to van't Hoff's law, the quantity Δp_{osm} can be substituted by the product $RT\Delta c$, where Δc denotes the difference in concentration of solute on the two sides of the membrane. Thus, instead of [4.51] we may write

$$J^m_{water} = -p_{water}(p_{hst} - RT\Delta c) \qquad [4.52]$$

The van't Hoff relation in this form is a good approximation if the membrane is perfectly impermeable for the solutes. In this case Δc denotes the total molar concentration difference, taking into consideration every solute. For biological membranes this requirement is generally not satisfied, because most of the ions or molecules are passing through the membrane to various degrees. This process is accounted for by introducing the *reflection constant* (δ), which is the quotient of two water fluxes. The numerator contains the flux produced by the semipermeable membrane for a given solute at a given concentration difference, and the denominator contains the flux measured if the membrane is perfectly impermeable for the solute at the same concentration difference. In the case of impermeable material $\delta = 1$, and for perfectly permeable substances $\delta = 0$ (these latter do not produce any osmotic pressure). With biological membranes, for most solutes $0 < \delta < 1$. On introduction of the reflection constant, [4.52] takes the following form:

$$J^m_{water} = -p_{water}\left(p_{hst} - RT \sum_{i=1}^{n} \delta_i \Delta c_i\right) \qquad [4.53]$$

where the summation must be carried out for every solute species in the system. The above reasoning holds exactly only for simple, homogeneous membranes. With more composite membranes the flux is a non-linear function of the osmotic pressure.

4.7.3. Active transport as a cross effect

In most thermodynamic systems, including biological systems, more than one thermodynamic forces are generally active. For instance, electric and chemical potential gradients may act in electrolytes (cf. section 4.6.2). Several currents may flow simultaneously in the systems. Thus in electrolytes not only material transport but also electric charge flow may take place. The flow of an extensive quantity is not only induced by the inhomogeneity of the corresponding intensive quantity (cf. Table 4.6), but in principle is influenced by all thermodynamic forces in the system. In the simple case when two fluxes (J_1, J_2) and two thermodynamic forces (X_1, X_2) exist, Onsager's relations can be written as

$$J_1 = L_{11}X_1 + L_{12}X_2$$
$$J_2 = L_{21}X_1 + L_{22}X_2$$

X_1 and X_2 are the thermodynamic forces *corresponding* to J_1 and J_2, respectively, and for this reason the factors L_{11} and L_{22} are called direct phenomenological coefficients. L_{12} and L_{21}, on the other hand, characterize the relations between the fluxes and

thermodynamic forces not associated with each other, and for this reason they are called the cross coefficients.

Many examples demonstrating cross effects might be mentioned, such as the thermodiffusion when a material flow is induced by temperature difference. The reverse process is also known. In this case a heat flow due to a chemical potential difference is obtained. In thermoelectric phenomena, heat flow is produced by electric potential differences, or a charge flow induced by temperature differences is obtained.

Cross effects may also occur in biological systems. In temperature sensation a temperature difference, and in pressure or sound sensation a pressure difference induces an electric charge displacement (receptor potential, cf. section 6.4.1). To generalize the above examples, it may be stated that any excitation is always associated with a change (gradient) in some intensive quantity, which results not only in the flow of the respective extensive quantity, but also in a charge displacement.

We shall next show that one of the most important and complex transport processes in the functions of cells, the active transport, may be regarded as a cross effect. It is a well-known fact that the life functions require concentration differences. In the case of sodium and potassium ions a more than tenfold concentration ratio exists between the extra- and intracellular spaces, and large concentration differences can be observed relative to other materials e.g. glucose or the amino acids. The concentration differences can naturally be kept constant only if the passive transport in the direction of the concentration gradient is accompanied by transport in the opposite direction, against the concentration gradient. This latter process is not spontaneous, but requires energy from the metabolic processes. Transport against the concentration gradient using the energies given by chemical processes is called *active transport.*

Let us consider as a rather simple case the transport of sodium ions and the metabolic processes associated with this process. Two thermodynamic forces act in this system: the *electrochemical potential gradient* ($\Delta\mu^e/\Delta x$) of the sodium-ion, and the *affinity of the energy-producing chemical reaction (A)* sustaining the active transport. The cross effect is manifested in the fact that the flux J of sodium ions is induced not only by the respective thermodynamic force, i.e. the electrochemical potential gradient, but is also influenced by the affinity of the chemical reaction. Consequently, we may write

$$J = -L_{11}\frac{\Delta\mu^e}{\Delta x} + L_{12}A$$

A direction is attributed to the flux, which means that J represents a vector. From this it follows that the other two expressions must necessarily be vectors too. The first term becomes a vector through the electrochemical potential gradient, which is multiplied by the scalar L_{11}. However, in the second term the affinity is a scalar, and thus it obtains its vectorial character through the factor L_{12}. The vectorial character of the second term expresses the situation that, in the case of active transport, the chemical reaction *induces a directed ion flux.* Thus, active transport can be attained

only in an *anisotropic medium*. Examples of such media are biological membranes composed of oriented molecules. The active transport results from the participation of these molecular structures.

Let us now compare passive and active transport from an *energetic* aspect.

Passive transport is a spontaneous, isothermal process during which the Gibbs free energy of a system consisting of a membrane and the solutions on the two sides of the membrane *decreases*, i.e. $\Delta G<0$. From the relation $\Delta G=\Delta H-T\Delta S$, a decrease in the Gibbs free energy may be due to either a decrease in enthalpy ($\Delta H<0$) or an increase in entropy ($\Delta S>0$). In the course of passive transport the internal energy of the system does not change considerably; there is usually no appreciable volumetric work, though because of the concentration equalization the entropy of the system increases (the ordering decreases). The decrease in the Gibbs free energy is mainly due to this latter circumstance.

In the course of *active transport* the ordering of the ions on the two sides of the membrane increases, which results in a decrease in the entropy of the transport system. Since the enthalpy does not change considerably in the process, the Gibbs free energy of the system *increases* due to the decrease in the entropy. Active transport can take place only if the strict transport system is associated with a process whose Gibbs free energy decrease can cover the above-mentioned increase. The process with decreasing Gibbs free energy associated with the transport is always some chemical reaction, in most cases the decomposition of adenosine triphosphate (ATP). In this latter case the Gibbs free energy decrease is a consequence partly of the decrease in the enthalpy and within this the internal energy, and partly of the increase in the entropy. The internal energy decreases in this case as a result of the rearrangement of the atoms in the course of the reaction, and the entropy increase follows from the increase in the disorder resulting from the decomposition. In the decomposition of ATP, the enthalpy decrease and the entropy increase depend sensitively upon the concentrations of other substances present in the medium (e.g. hydrogen ion, magnesium ion). In the living organism the two factors participate nearly equally in the change of the Gibbs free energy.

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5. BIOMEDICAL ELECTRONICS

A large variety of electronic devices are used in medical practice and scientific research. In the narrower sense these are primarily devices employed in the diagnostic and therapeutic practice in direct contact with the patients (medical electronics). However, in a broader sense all electronic instruments used in medical laboratories or in scientific research, e.g. in chemical analysis or structural investigations, have a bearing on bioelectronics.

This chapter deals mainly with the general physical and technical bases necessary for an understanding of the function of the devices used in medical electronics; however, their general character means that they can serve as the basis of more wide-ranging information.

In the discussion of the subject technical details are avoided, only the basic principles, and the larger functional units will be dealt with, as far as necessary for an understanding of their function.

5.1. Signals as information carriers

1. Signals in general, and their role in medicine. The organism, and within it the individual cells, tissues and organs (in brief any biological system), continually interacts with the surroundings and with other systems. It is especially easy to demonstrate the interaction between the organism and the external world. In one direction of this interaction the organism perceives effects arriving from the external world and processes the information content of these effects. This is the basis of the adaptation of the organism to the surroundings and its changes. The other direction of the interaction is connected with the fact that the life functions are accompanied by various phenomena which can be observed and recorded and which supply information that may be processed by the environment. The information is always carried by some physical quantity, e.g. light, sound reaching the organism or heat emitted by it. *A quantity (or its change) carrying or storing information is called a signal.* However, the definition means that the concept of a signal is more general than indicated by the above examples. For a presentation of the more general concept of signals, it is worthwhile to mention some additional examples. For instance, the electric potential

generated on cardiac action yields valuable information on the activity of the myocardium; similarly, the blood sugar concentration is an important indicator of the metabolism; the DNA base sequence carries genetic information; and so on.

The signal, or information, may refer to the state of the system, to some process, some phenomenon, etc. One such state parameter is the body temperature. The signal associated with this is continuous, and its magnitude is approximately constant. As an example of periodic processes, the ECG signal associated with the heart function may be mentioned. An instance of a single process is the stimulation process induced by an electric pulse, accompanied by an electric signal of characteristic form. A random sequence of individual events (stochastic process) is the γ-radiation emitted by some radioactive preparation. The voltage pulse signals of the scintillation counter correspond to individual γ-photons.

Of the quantities used as signals, electric signals can relatively easily be processed by electronic devices. For this reason, as a first step the originally non-electric signals are transformed to electric signals. During processing further transformations of the electric signal may become necessary. However, it is an absolute requirement that the information content of the signal must not change during its transformation.

Two types of signal transformation are known: *analogue* and *digital* transformation. In the former case the time course of the transformed signal is similar (analogous) to that of the original signal. Thus, the electrocardiogram represents a graphically recorded analogue signal of the action potential changes of the myocardium. In the case of a digital transformation, elements of a symbol system, in most cases numbers, are unambiguously assigned to the instantaneous values of the signal. This happens, for instance, when a change in the myocardial action voltage is stored as a series of numerical values in the memory of a computer.

The methods and devices applied in biomedical electronics can be classified according to medical aspects in the following way:

(a) the processing of the signals associated with the state or function of the system under investigation (cell, organ, organism), with the purpose of obtaining diagnostic or scientific information (e.g. electroencephalography, thermography);

(b) a signal produced by an electronic device is introduced into the system to be investigated to obtain information on its structure, state or function (e.g. echocardiography);

(c) a signal produced by an electronic device is introduced into the system to influence its state or function (e.g. high-frequency heat therapy).

Figures 5.1*a*, *b* symbolize diagnostic applications, and Fig. 5.1*c* therapeutic ones. The arrows in the diagram indicate the direction of information flow, which is always associated with some energy transport. Signal power is mentioned in this sense throughout this chapter.

2. The concept of decibel. Signals can be compared by means of the ratio of their powers. Frequently, the logarithm of this ratio to the base ten is used, or this value

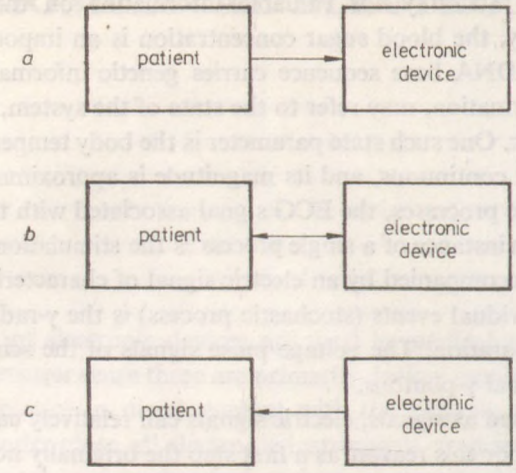

Fig. 5.1. Typical cases of signal energy flow

multiplied by 10. The scales thus obtained are called the bel and decibel scales (denoted by B or dB). Let us denote the reference power by P_1 and the power in question by P_2; the relations used for their comparison are then

$$n(\mathrm{B}) = \log \frac{P_2}{P_1} \mathrm{B} \quad \text{or} \quad n(\mathrm{dB}) = 10 \log \frac{P_2}{P_1} \mathrm{dB} \qquad [5.1]$$

The bel scale is too large for practice, therefore the decibel scale is frequently used. If the signal is an electric one, the calculations may involve voltage instead of power ratios. In this case

$$n = 20 \log \frac{U_2}{U_1} \mathrm{dB} \qquad [5.2]$$

where U_2/U_1 denotes the voltage ratio.

[5.2] can easily be obtained from [5.1] if the relation $P = U^2/R$ is considered and if it is assumed that the two signal powers or signal voltages appear on the same resistance R.

3. Signal-to-noise ratio. The signal to be processed is frequently accompanied by some identical or similar signal produced by the signal source or the surroundings. For instance, the observation of cardiac sounds may be disturbed by various other sounds produced either by the patient or by the environment. This accompanying signal disturbs the information content of the signal to be processed (disturbing signal, noise). The signal-to-noise ratio is usually expressed by the ratio of the signal voltage (U_{sig}) to the noise voltage (U_{noise}), or on the decibel scale.

5.2. Electronic systems

Electronic systems are built up from combinations of basic elements for certain purpose. The more important elements are voltage or current sources, constant or variable resistors and capacitors, induction coils, rectifiers (diodes), amplifier units (transistors and not frequently electron tubes), displays (e.g. cathode-ray tubes, liquid crystals, LEDs, etc.), sensors and transducers (photocells, thermoelements and so on). Any desired function can be obtained by various combinations of the elements; for this reason the user need not study the working of an electronic system in detail; it is sufficient to know its *functional units* and their interrelation. The functional units are frequently called *blocks,* which are usually represented by rectangles with their function written inside them. In this way, instead of by means of a large number of details, an electronic system can be depicted by a *block diagram,* which permits the study of its operation too.

Semiconductor technology has led to a considerable decrease in the size of electronic equipment. In the case of *integrated circuits (IC)* every unit of a block can be developed on a single semiconductor (e.g. silicon) plate: several transistors, diodes, capacitors and resistors and their connections. From a given *IC* blocks of different electric functions can be formed.[1] With up-to-date technology, several ten thousand units can be placed on a surface of 1 mm^2. As a result of the development of microelectronics, not only the dimensions of the devices but also their power consumption has been decreased by several orders of magnitude. From the medical viewpoint beside the decrease in size the further advantage is that an ever increasing number of devices are portable and can be operated independently of the electric mains network, which is important from the aspect of safety, too.

Certain combinations of elements play a fundamental role in the individual blocks; these are basic electronic circuits. In the following sections some units and basic electronic circuits will be considered in more detail.

5.2.1. Electronic components and basic circuits

1. The diode has two electrodes, and can be used mainly as a rectifier. Its resistance is low for one polarity of the applied voltage *(forward voltage)*, whereas it is high for the other polarity *(reverse voltage)*. Such a case is demonstrated in Fig. 5.2, which depicts the voltage (U)-dependence of the diode current (I) (diode characteristic). The left-hand side of the diagram shows the symbol of the diode, with the notation of the polarity of the forward voltage.

[1] The degree of integration may be characterized by the number of transistors on the semiconductor plate (chip). In the SSI (Small Scale Integration) technology a single silicon plate with a surface of a few tenths of a cm^2 contains not more than 50 transistors, in the VLSI (Very Large Scale Integration) technology the number of transistors is already 10^4–10^5.

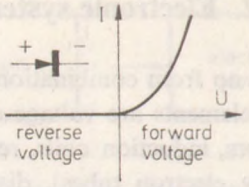

Fig. 5.2. Symbol and characteristic of the diode

2. The transistor consists of three semiconductor layers with three electrodes: *emitter* (*E*), *base* (*B*) and *collector* (*C*). It operates with two circuits (Fig. 5.3): the *base circuit* with the base voltage source U_B, and the *collector circuit* with the supply voltage source (U_S). These two circuits are not independent of each other, and this is the basis of the transistor application.

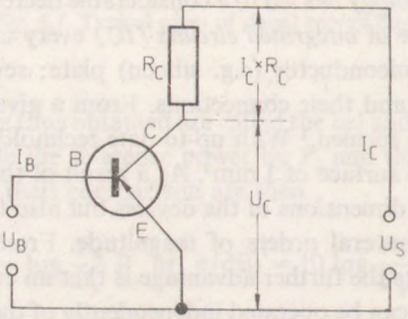

Fig. 5.3. Diagram relating to application of the transistor

(a) The transistor may be used as an *amplifier*. The voltage to be amplified is applied to the base circuit as a change of the base voltage. The base current and also the collector current intensity change proportionally to the change of the base voltage. Simultaneously with this latter current intensity change, the voltage on the resistor R_C in the collector circuit (the load resistor) will change. The emitter–collector unit of the transistor and the resistor R_C together form a voltage divider which gives on R_C a part of the voltage U_S which is proportional to I_C: $I_C R_C$. If this increases, the collector voltage U_C decreases. If the change in the base voltage represents a signal voltage, the change of U_C will appear as a proportionally increased signal voltage. Their ratio is called the *voltage* gain. The signal power can be calculated from the signal voltage across a resistance if the value of the resistance is known. This holds for both the resistor of the base circuit and the load resistor in the collector circuit. Their ratio is the *power gain*.

(b) The transistor can be used as a *switching unit* too. This is based on the property that the emitter and the base operate together as a diode. Collector current will flow

in a transistor only with a forward base voltage; with a reverse base voltage, the intensity of the collector current is zero. Consequently, the transistor may be used as an electronic switch which, depending upon the base voltage, disconnects or connects the emitter and collector electrodes. If suitable auxiliary circuits are applied, the switching occurs at a preset base voltage.

3. Voltage division. Potentiometer. If some voltage is applied to two or more resistors connected in series (Fig. 5.4*a*), the ratio of the voltages on the resistors correspond to the ratio of their resistances (e.g. $U_1/U_2=R_1/R_1$). This is valid for both direct and alternating current, and the resistances may be not only ohmic, but also inductive and capacitive (cf. the *RC* circuit below). One frequently used solution is depicted in Fig. 5.4*b*. The device, called a potentiometer, allows a continuously variable voltage division by movement of the sliding contact *C*. The knobs regulating amplification, light intensity, sound intensity, etc., on electronic devices are usually the rotatable knobs of potentiometers.

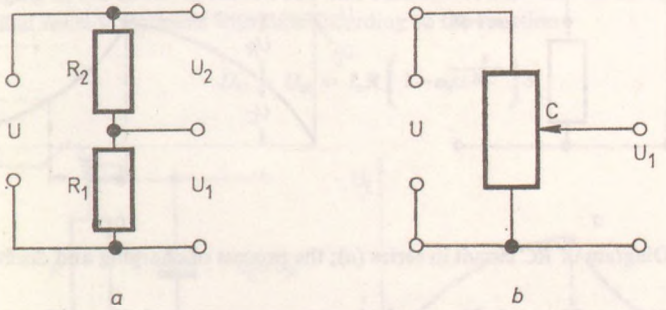

Fig. 5.4. Voltage division

4. LC circuit. Circuits consisting of a coil of inductance *L*, and a capacitor of capacitance *C* are called *LC* circuits (Fig. 5.5). In these circuits electromagnetic oscillations can be produced if, for instance, an alternating current is applied to points 1 and 2. For this reason such circuits are frequently called *oscillator circuits*. An *LC* circuit will be resonant to the exciting voltage if its frequency is equal to the resonance

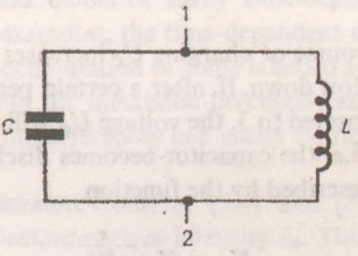

Fig. 5.5. LC circuit

frequency (ν_0) of the circuit. ν_0 can be expressed in terms of the characteristic data of the circuit by the relation

$$\nu_0 = \frac{1}{2\pi\sqrt{LC}} \qquad [5.3]$$

If either L or C or both are varied, the resonance frequency of the circuit can be changed, i.e. the LC circuit can be tuned. (The applications of this circuit will be discussed in section 5.4.)

5. RC circuit. Resistor R and capacitor C are connected in series, form a *series RC circuit*. When a constant voltage U_S is applied to the RC circuit (by short-circuiting 1 and 2 in Fig. 5.6a), a voltage U_C proportional to the charge Q flowing onto the capacitor will appear. The intensity I of the charging current is proportional to the

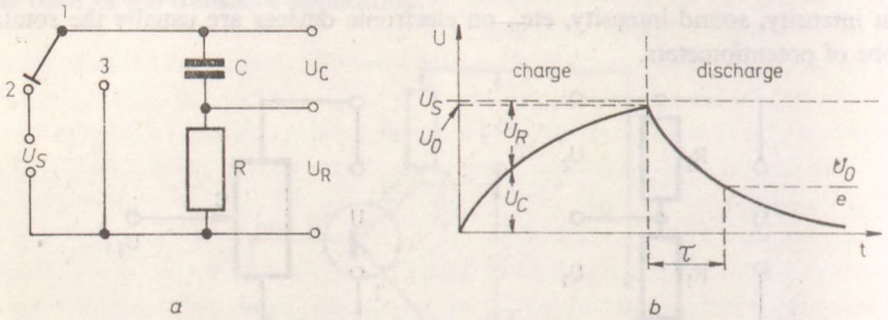

Fig. 5.6. Diagram of *RC* circuit in series (*a*); the process of charging and discharging (*b*)

difference between U_S and U_C, and decreases exponentially with time (t) during charging. Since the voltage U_R across the resistor R is proportional to the current, U_R will also decrease exponentially as a function of time:

$$U_R = U_S e^{-\frac{t}{RC}} \qquad [5.4]$$

As the sum of U_R and U_C at every moment is equal to U_S, we have

$$U_C = U_S\left(1 - e^{-\frac{t}{RC}}\right) \qquad [5.5]$$

which means that in the course of charging U_C increases rapidly at first, but subsequently the increase will slow down. If, after a certain period of charging, 1 and 2 are disconnected, and 1 is connected to 3, the voltage U_C will decrease from the value U_0 attained during charging, i.e. the capacitor becomes discharged through the resistor R. The discharge can be described by the function

$$U_C = U_0 e^{-\frac{t}{RC}} \qquad [5.6]$$

On discharge, $U_R+U_C=0$ at every moment, and consequently $U_R=-U_C$. The process of charging and discharging is demonstrated in Fig. 5.6*b*.

The product $\tau=RC$ characteristic of the *RC* circuit has the dimension of time, and is the *time constant* of the circuit. According to [5.6], the time constant is the time required for the voltage of the charged capacitor to decrease by the factor *e*.

[5.5] can be derived in the following way. From Ohm's law:

$$U_R = IR \text{ and } U_S - U_C = \frac{dQ}{dt}R$$

It follows from the definition of the capacitance that $dQ=CdU_C$, so that the above equation can be rewritten in the form

$$U_S - U_C = \frac{dU_C}{dt}RC$$

Integration of this differential equation leads to [5.5]. [5.6] can be derived by similar reasoning.

Figure 5.7 depicts a *parallel RC circuit*. Mention is made only of the practically interesting case when the charging of the circuit is carried out with constant current. In this case the voltage across the capacitor and resistor increases with time according to the function

$$U_C = U_R = I_0R\left(1-e^{-\frac{t}{RC}}\right) \qquad [5.7]$$

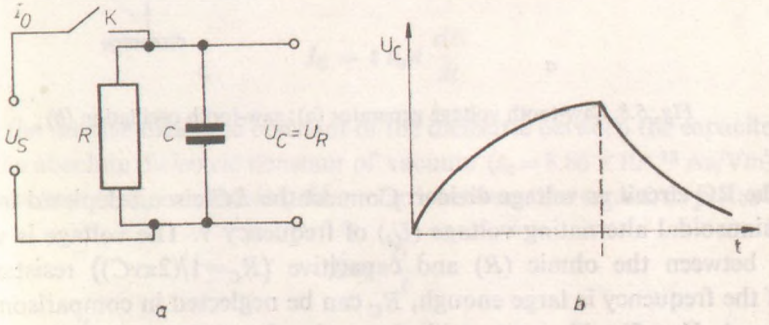

Fig. 5.7. Diagram of a parallel *RC* circuit

The *RC* circuit has many applications in electronic systems and some of these will be discussed below. Here it is stated only that the charging and discharging of the *RC* circuit is a good model of many time-dependent exponential processes occurring in Nature. As examples, the time-dependent decrease in activity of radioactive preparations, the accumulation of some labelled substance in an organ or the release of this substance in the metabolic processes, and the equalization of temperature and concentration differences can easily be modelled with *RC* circuits.

6. Saw-tooth wave generator. Consider [5.4] and [5.5]. These also hold if the circuit is charged with constant current intensity I_0. This is the situation in the initial stage of the charging, when U_C can be neglected relative to U_S; in this case $I_0=U_S/R$.

It is clear that in this initial stage of charging, i.e. as long as $t \ll RC$, the charging of the capacitor is a linear function of time:

$$U_C = \frac{I_0 t}{C} \qquad [5.8]$$

This linear charging process is used to produce saw-tooth voltage. The generator is depicted in Fig. 5.8. The capacitor voltage increases uniformly up to a certain value; when this has been attained, the switching circuit S (cf. section 5.2.1) discharges the capacitor, after which the process is repeated. Saw-tooth wave generators are used, for instance, in cases when various periodic phenomena are displayed by cathode-ray oscilloscopes. In these cases the horizontal (time) axis of the oscilloscope is given by the linearly increasing range of the saw-tooth voltage (cf. section 5.3.2).

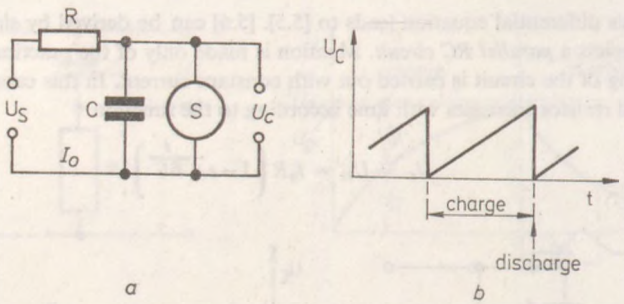

Fig. 5.8. Saw-tooth voltage generator (*a*); saw-tooth oscillation (*b*)

7. The RC circuit as voltage divider. Connect the *RC* circuit depicted in Fig. 5.9*a* with a sinusoidal alternating voltage (U_i) of frequency ν. The voltage is vectorially divided between the ohmic (R) and capacitive ($R_C = 1/(2\pi\nu C)$) resistances (Fig. 5.9*b*). If the frequency is large enough, R_C can be neglected in comparison to R, i.e. $U_C = 0$ and $U_R = U_i$. However, with decreasing frequency, U_C increases, whereas

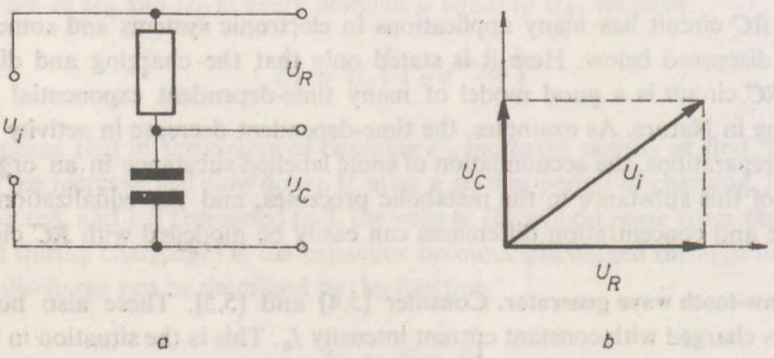

Fig. 5.9. Voltage division with an *RC* circuit

U_R decreases. The freqeuncy ν_l for which $R=R_C$, i.e. $R=1/(2\pi\nu_l C)$ and consequently

$$\nu_l = \frac{1}{2\pi RC} \qquad [5.9]$$

is the *limiting frequency*. At this frequency $U_R=U_C$ and, as a special case of Fig. 5.9*b*, both are equal to $U_i/\sqrt{2}$. This means that at the limiting frequency the signal-power transmission is -3 dB, or the decrease of the signal power (the attenuation) is 3 dB. The consequence of further frequency decrease will be further attenuation. A frequency decrease of one octave (a twofold factor) results in an attenuation of 6 dB.

8. Capacitive current. In conductors electric current is generated by charge flow. Though in the case of ideal insulating dielectrics there is no charge movement, one may e.g. refer to the current flowing through a capacitor. The variation of the electric field strength is also equivalent to current since it creates a magnetic field in the same way as the so-called conduction current in conductors. The current due to field strength variation is called capacitive current.

The intensity of capacitive current (I_C) is proportional to the change of the electric field strength in unit time (dE/dt) and to the area (A) of the capacitor plate

$$I_C = \varepsilon\,\varepsilon_0 A \frac{dE}{dt} \qquad [5.9a]$$

where ε is the relative dielectric constant of the dielectric between the capacitor plates and ε_0 is the absolute dielectric constant of vacuum ($\varepsilon_0=8.86\times10^{-12}$ As/Vm). Let us take into account that the electric field strength between the capacitor plates is

$$E = \frac{U}{d}$$

and the capacitance (C) of the capacitor is

$$C = \varepsilon\,\varepsilon_0 \frac{A}{d}$$

U denotes the voltage and d the distance between the plates. Thus after conversion of [5.9a] the expression

$$I_C = C\frac{dU}{dt} \qquad [5.9b]$$

is obtained for the capacitive current (cf. section 6.1.3: capacitive current).

Thus in the case of a series *RC* circuit too we are dealing with a closed circuit: in the wires and the resistor conduction current, in the dielectric of the capacitor (or even in vacuum) capacitive current flows.

If the dielectric is not an ideal insulator but it has a finite resistance and conduc-

tivity (dissipative dielectric), the capacitor itself shows the behaviour of a parallel *RC* circuit as part of the electric circuit in question. The above-mentioned are valid in both direct and alternating current circuits.

9. Filter circuits. The *RC* circuit properties discussed in the previous point permit signal analysis according to frequency. As demonstrated in Fig. 5.10, voltage division can be performed with *RC* circuits in two ways, giving two means of frequency filtering. In case *a*, the *RC* circuit lets through practically all frequencies larger than the limiting frequency, whereas the frequencies below the limit are cut off. Diagram *b* depicts the reverse process. Thus, the *RC* circuit may be used as a filter circuit operating either above or below the frequency limit. The right-hand side of Fig. 5.10 depicts the transfer characteristics; the abscissa shows the frequency, while the ordinate gives the transfer expressed in dB. Zero dB means transfer without attenuation. The filter circuits are characterized not only by the limiting frequency but also by the cut-off slope, the slope of the oblique straight line. In the given case its (absolute) value is 6 dB/octave.

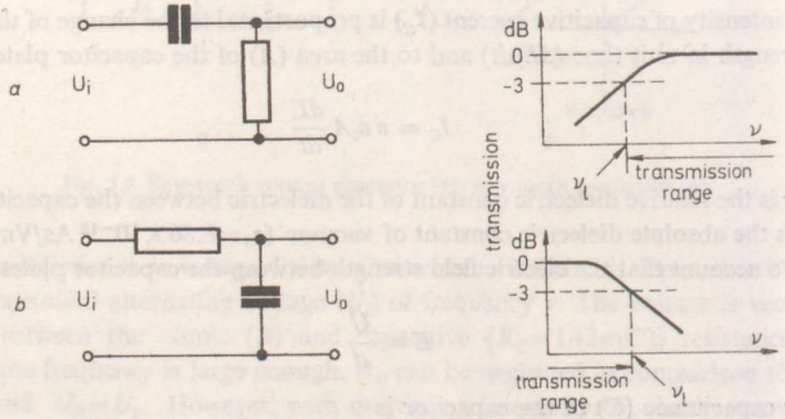

Fig. 5.10. RC filter circuits and their characteristics

With a combination of elements with frequency-dependent impedances (*L* and *C*) and resistances it is possible to construct filter circuits with any transfer characteristics: for instance, filter circuits transmitting in a determined frequency range. It is of interest to note that the chest behaves with respect to the heart sounds as a low pass acoustic filter with a cut-off slope of approximately −12 dB/octave. Consequently, with a suitable series of high pass filters the heart sound can be recorded according to frequency bands (phonocardiography). The electric voltage signals accompanying the function of the central nervous system can similarly be processed according to frequency bands (electroencephalography).

10. Ratemeters. A common problem is the determination of *pulse frequencies*, e.g. the particle flux in nuclear radiation. These types of measurements may be carried out with a ratemeter, consisting essentially of *RC* circuits (Fig. 5.11). It is a basic condition of the measurement that every pulse input should produce equal charges on the capacitor C across the resistor R. (A diode prevents the reverse flow of charge.) This condition can be achieved by forming signals of identical amplitude and shape prior to the input. The capacitor C together with the resistor R' of the voltmeter U constitute an *RC* circuit whose time constant is larger than the consecutive time intervals between the individual pulses. Under these circumstances U_C is proportional to the pulse frequency ν, and can be read off the voltmeter.

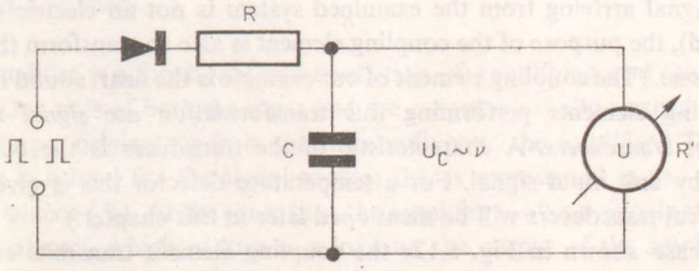

Fig. 5.11. Diagram of the ratemeter

11. Coupling elements. Coupling circuits. These elements connect the patient (or more generally the system to be investigated or influenced) with the electronic device; i.e. they ensure the flow of the signal energy in the required direction. Figure 5.12 is similar to Fig. 5.1, the only difference being that the coupling elements are shown as blocks separate from the electronic device. Various signals may occur

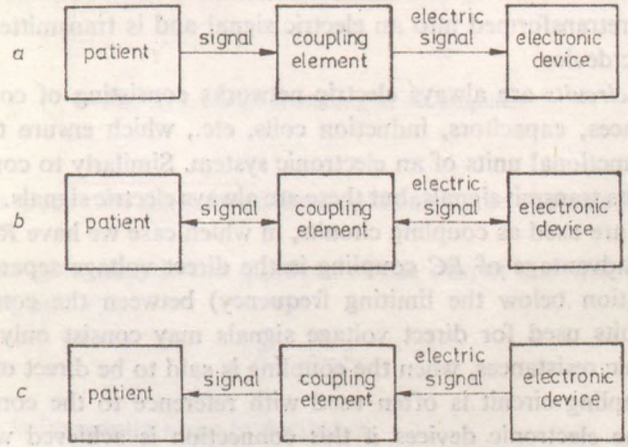

Fig. 5.12. Functions of the coupling elements

between the coupling element and the examined system, but always electric energy is transmitted between the coupling element and the electronic device.

Let us consider first the case depicted in Fig. 5.12*a*, where the processing of signals arriving from the patient is demonstrated. This signal may be some electric voltage, associated for instance with the functioning of some organ. The purpose of the coupling element in this case is the production of an electric contact between the patient and the signal-processing system. Such coupling elements are called *electrodes*. However, all electroanalytical auxiliary devices giving a voltage depending on the ionic milieu (e.g. ion-selective electrodes) are also called electrodes. Their application allows the fast, exact and easy determination of the concentrations of inorganic and organic substances in the body fluids.

If the signal arriving from the examined system is not an electric one (e.g. the heart sound), the purpose of the coupling element is also to transform the signal into an electric one. (The coupling element of our example is the heart sound microphone.) The coupling elements performing this transformation are *signal transformers, detectors* or *transducers*. A characteristic of the transducer is the output voltage produced by unit input signal. For a temperature detector this is given in mV/K. (Some typical transducers will be mentioned later in this chapter.)

In the case shown in Fig. 5.12*c* the coupling element transmits energy to the patient. This energy transmission may also be achieved without energy transformation (e.g. in the case of electric excitation). The coupling element here too is called an electrode. An example of energy coupling with energy transformation is the earphone, which in audiometry transmits to the patient electric power of audiofrequency transduced into sound.

Figure 5.12*b* depicts a coupling element functioning in both directions. Such two-way transducers are used in ultrasound diagnostics (see section 5.4.2). By means of this transducer, electric energy of ultrasound frequency is transformed into ultrasound, which is radiated into the system under examination, and additionally the reflected ultrasound is retransformed into an electric signal and is transmitted into the processing electric device.

Coupling circuits are always electric networks consisting of connecting wires, ohmic resistances, capacitors, induction coils, etc., which ensure the connections between the functional units of an electronic system. Similarly to coupling elements coupling circuits transmit signals, but these are always electric signals. Quite frequently *RC* circuits are used as coupling circuits, in which case we have *RC* or capacitive coupling. An advantage of *RC* coupling is the direct voltage separation (or more exactly separation below the limiting frequency) between the connected blocks. Coupling circuits used for direct voltage signals may consist only of connecting wires and ohmic resistances, when the coupling is said to be direct or galvanic. The expression coupling circuit is often used with reference to the connection of the patient and the electronic devices if this connection is achieved with a network

consisting of electrodes, electric wires and other connecting units. The coupling circuits transmitting electric power to the patient are frequently termed *patient circuits*.

5.3. Basic electronic functions

5.3.1. Amplifiers and their amplification

The power of the electric signal carrying the information is usually not high enough for further processing. For this reason signals must be amplified by electronic devices called amplifiers.

1. The amplifier is a functional unit consisting of amplifying and other elements (Fig. 5.13). (One pole of both the input and the output is usually earthed to decrease the noise voltages originating from the surroundings of the amplifier.) The input of the amplifier is a load for the signal source; this is represented in the diagram by the input resistance (R_i). At the same time the amplifier is also a signal source for the next functional unit, which in turn is a load on the output of the amplifier (R_o in the diagram.)

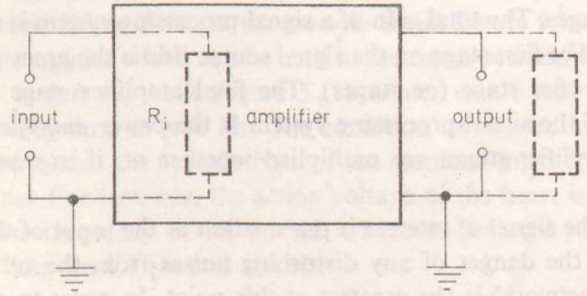

Fig. 5.13. Diagram relating to the amplifier

2. Gain. In a given case the gain of the amplifier may be characterized in various ways.

(a) *The power gain* (K_P) is the quotient of the output (i.e. amplified) and the input powers (P_o and P_i):

$$K_P = \frac{P_o}{P_i} \qquad [5.10a]$$

The power gain is frequently given in decibels:

$$K_P \text{ (dB)} = 10 \log K_P \text{ dB} \qquad [5.10b]$$

(b) Instead of power, the signal can be characterized by its voltage; therefore, the *voltage gain* (K_U) too is used, which is the quotient of the output and input signal voltages (U_o and U_i):

$$K_U = \frac{U_o}{U_i} \qquad 5.11]$$

(c) The relation between the voltage and the power gain is given by the simple relation

$$K_P = K_U^2 \frac{R_i}{R_o} \qquad [5.12a]$$

or, expressed in decibels:

$$K_P \text{ (dB)} = \left(20 \log K_U + 10 \log \frac{R_i}{R_o}\right) \text{dB} \qquad [5.12b]$$

To express only the variation of the gain (in the regulation of gain) we may use the equation

$$K_P \text{ (dB)} = 20 \log K_U \text{ dB} \qquad [5.12c]$$

As an example, it may be mentioned that the gain required with an ECG apparatus amounts to 60 dB, which corresponds to a power gain of 10^6 or a voltage gain of 10^3.

3. Amplifier stages. The total gain of a signal-processing system is usually attained in several stages. The first stage on the signal source side is the *preamplifier*, followed by the main amplifier stage (or stages). The final amplifier stage before the last functional unit of the signal-processing system is the *power amplifier*. The gains of the individual amplifier stages are multiplied together or, if expressed in decibels, added.

The power of the signal of interest is the smallest at the input of the preamplifier, and consequently the danger of any disturbing noises from the surroundings (e.g. from the electric network) is the greatest at this point. In order to eliminate noise, the preamplifier requires the most careful construction. For similar reasons all the circuits used to manipulate the signal are placed at a higher signal level between the consecutive amplifier stages. The filter circuits already mentioned, or the gain regulators to be discussed later are placed between these stages.

4. Regulation of gain. The regulation may be continuous or stepwise, using a voltage divider resistance-chain for instance. Figure 5.14 illustrates the continuous regulation of gain. Stepwise regulation, on the other hand, can be performed with a decadic divider. For the different switch positions one-tenth, one-hundredth, one-thousandth, etc. of the input voltage are obtained at its output. Instead of the value of the voltage division, the value of the attenuation expressed in dB is given frequently, e.g. 0, −20, −40 dB, etc.

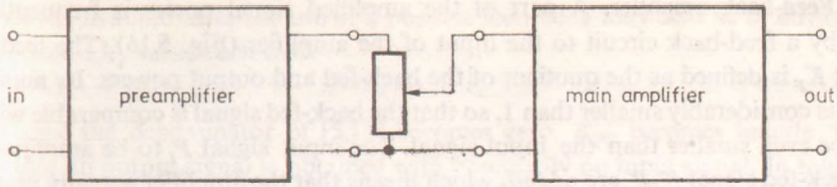

Fig. 5.14. Diagram relating to gain regulation

5. Transfer characteristics. It is a basic condition in amplification that it must be frequency-independent in the whole frequency range. The meaning and importance of this condition become obvious if it is considered that most signals cannot be described by a single harmonic oscillation (sine curve). However, any periodic or aperiodic signal can be considered as the sum of harmonic oscillations. Hence, it is not sufficient to characterize a signal by only one frequency; instead, a series of frequencies or a frequency interval (frequency band) is required. This series of frequencies, or band, is called the total frequency range of a signal (cf. Table 6.4). If the above condition were not satisfied, the various frequency components of the signal would be amplified to various degrees and, as a consequence, the shape of the signal would become distorted. The frequency-dependent description of the amplification of the amplifier gives the *transfer characteristics* (Fig. 5.15). The frequency range within which the gain is independent of the frequency (or more exactly where the frequency dependence remains below 3 dB) is the *transfer band.* Figure 5.15*a* shows the characteristics of an amplifier which can be used in the range between the low- and high-frequency limits ν_l and ν_h, while Figure 5.15*b* relates to a direct voltage amplifier, whose low-frequency limit is zero Hz. The condition mentioned in the introduction can also be stated in that the *transfer band* of the amplifier must cover the frequency range of the signal. For instance, the action voltage of the heart is a nearly periodic signal with a base frequency of 1.1–1.3 Hz, whose undistorted processing requires a transfer band between 0.1 Hz and 100 Hz.

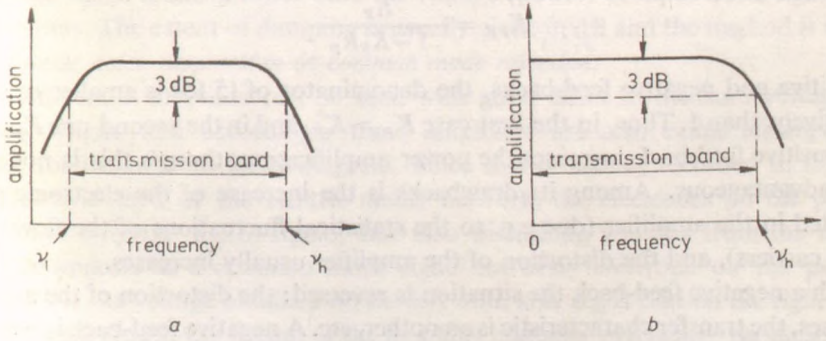

Fig. 5.15. Transfer characteristics of the amplifier

6. Feed-back amplifier. A part of the amplified signal power is frequently fed back by a feed-back circuit to the input of the amplifier (Fig. 5.16). The feed-back factor K_F is defined as the quotient of the back-fed and output powers. Its numerical value is considerably smaller than 1, so that the back-fed signal is comparable with, or may be even smaller than the input signal. The input signal P_i to be amplified and the back-fed signal $K_F P_o$ are added, which means that the amplifier actually amplifies the resultant power

$$P = P_i + K_F P_o \qquad [5.13]$$

Thus, the power of the output signal can be rewritten as

$$P_o = K_P P \qquad [5.14]$$

where K_P denotes the power amplification of the amplifier without feed-back.

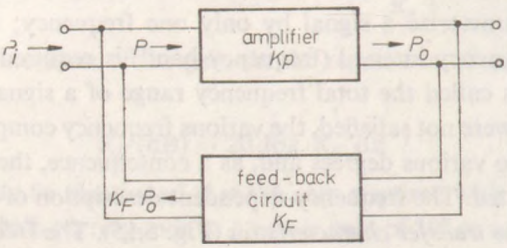

Fig. 5.16. Amplifier with feed-back

The result of the signal summation (interference) on the input depends upon the phase relations of the interfering signals. Of the many possible cases, we shall discuss here only two practically important extreme cases, i.e. when signals of identical or opposite phases are added. In the case of identical phases, the feed-back factor is considered to be a positive quantity ($K_F > 0$); this is a *positive feed-back*. In the opposite case, the feed-back is regarded as negative ($K_F < 0$), and this is a *negative feed-back*. Accordingly the power gain (K_{PF}) of the feed-back amplifier, defined by the quotient P_o/P_i, is

$$K_{PF} = \frac{K_P}{1 - K_F K_P} \qquad [5.15]$$

In positive and negative feed-backs, the denominator of [5.15] is smaller or larger, respectively, than 1. Thus, in the first case $K_{PF} > K_P$, and in the second one $K_{PF} < K_P$.

A positive feed-back increases the power amplification, though this is not necessarily advantageous. Among its drawbacks is the increase of the electronic noises generated in the amplifier (due e.g. to the statistical fluctuations of the flow of the charge carriers), and the distortion of the amplifier usually increases.

With a negative feed-back the situation is reversed: the distortion of the amplifier decreases, the transfer characteristic is smoother, etc. A negative feed-back is generally used to improve or modify the properties of the amplifier.

However, in some cases the use of a positive feed-back may also be of advantage. Let us select a K_F value such that

$$K_F K_P = 1 \qquad [5.16]$$

In this case the denominator of [5.15] becomes zero, K_{PF} becomes infinite, which means that an output signal is obtained with practically no input signal. In this case, however, we are no longer dealing with an amplifier, but with an *oscillator*, which in practice is used as a special electronic energy source (cf. section 5.3.3).

7. **The differential amplifier** has two inputs and one output (Fig. 5.17). The output signal voltage is proportional to the difference between the two input voltages:

$$U_o = K_U(U_{i1} - U_{i2}),$$

where K_U is the voltage amplification. The amplification can be expressed in decibels as well. The use of differential amplifiers renders possible the enhancement of the signal-to-noise ratio in cases when noise signals of common mode (identical shape, amplitude, phase) are superimposed on the signals to be amplified.

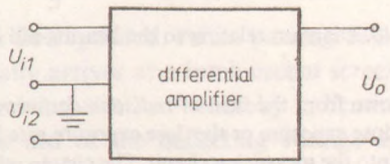

Fig. 5.17. Diagram of the differential amplifier

If the noise signals on the two inputs of the ideal differential amplifier were to be completely of common mode, their difference would be always zero, and the amplified output signal would not contain at all a component from this noise voltage. In practice this can never be completely fulfilled, the differential amplifier damps the noise signals of common mode to a great extent only, thus the output voltage (power) of the noise signal is much lower than the voltage (power) of input noise signals on the two inputs. The extent of damping is usually given in dB and the method is called *common mode noise suppression* or *common mode rejection.*

The differential amplifier can be used with good effect to measure voltages of biological origin, and accordingly these amplifiers are also called *bioamplifiers.* Consider for instance an ECG diagram. Since this is usually recorded in the disturbing electric field of the electric mains network, the electrodes on the patient transmit not only the useful signal, but also disturbing voltages from the mains. This noise appears as a common mode signal for every electrode on the patient. Thus, if U_{i1} is the voltage obtained on the left arm, and U_{i2} is that on the right arm, the signal measured at the output of the amplifier contains practically no noise from the mains network. A voltage of biological origin may also be a noise signal: for

instance, on the electrodes placed on the skull to measure the action potentials associated with the functions of the brain one can observe not only the useful signal, but also the action voltage of the heart, which appears on the EEG electrodes as a common mode signal.

8. Examples of the application of amplifiers. In the previous section we have frequently referred to medical applications. In this section two applications are described.

(a) Figure 5.18 depicts the basic construction of a hearing-aid device. A miniaturized microphone (M), as a transducer, detects the input sound power P_i. The amplified audiofrequency electric power is transformed by an equally small earphone (E) into acoustic power (P_o), which is irradiated into the ear of the patient. The hearing-aid operates properly only when the value of the acoustic amplification as defined by the ratio P_o/P_i is the same as the hearing loss. Of course, the electric amplification must be larger than the acoustic amplification, since the transformation losses in the microphone and in the earphone must be accounted for.

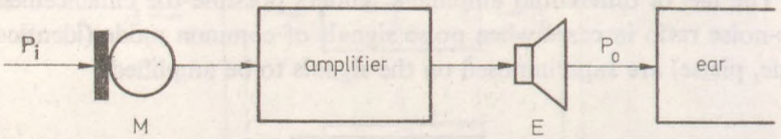

Fig. 5.18. Diagram relating to the hearing-aid device

(b) Another example is shown from the field of *radiation dosimetry* (Fig. 5.19; cf. section 2.14). Depending upon whether the dose exposure or the dose exposure rate is to be measured, a capacitor C or a resistor R is connected into the measuring circuit. The charge released in the measuring chamber either charges the capacitor to a degree proportional to the exposure, or a current with intensity proportional to the exposure rate will flow through the resistor. Consequently, the voltage over the capacitor or the resistor will be an analogue signal of the exposure or exposure rate; after amplification, this will be shown by the voltmeter or recorded by the recorder.

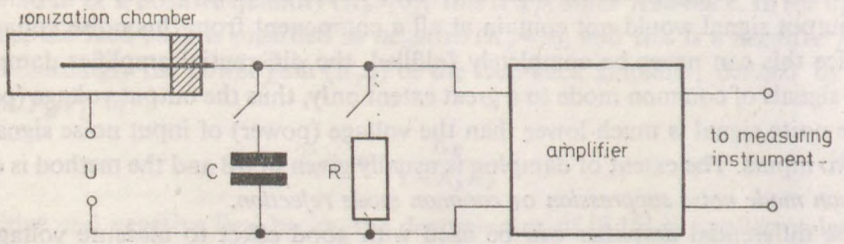

Fig. 5.19. Diagram relating to the measurement of dose and dose rate with an ionization chamber

5.3.2. Displays and recorders

The function of the last unit of the signal-processing systems is usually to display the signal for immediate use or to record it. This may mean the display of the instantaneous or steady signal value, but it may also result in a two-dimensional image or

a diagram demonstrating the course of a process in time. In the following section some of the more frequent methods of the display and the recording of two-dimensional images and time processes will be discussed.

1. The cathode-ray tube is an electronic device used to display time processes graphically and to produce two-dimensional images (Fig. 5.20). Similarly to TV image tubes, the cathode-ray tube consists of an electron source (hot cathode) and an electrode system (E) allowing the production of a narrow electron beam (cathode-

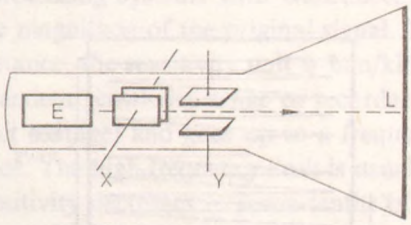

Fig. 5.20. Diagram of the cathode-ray tube

ray) whose intensity can be regulated. After passing two deflecting electrode pairs (X and Y), the beam finally arrives at a luminescent screen (L). The screen luminesces in response to the arriving electrons, thereby indicating the spot of the incident electron beam. With the aid of the deflecting voltage applied to the plate pairs, the cathode-ray can be deflected to any point of the screen. (This deflection can be accomplished not only with electric but also with magnetic fields.)

(a) Time processes can be displayed by applying a saw-tooth voltage to the deflecting plate pair X. As a result (during the rise of the voltage) the cathode-ray travels across the screen, which represents the time axis. The voltage signal of the studied time process is superimposed on this motion. This latter signal is applied to the deflecting plate pair Y. If the studied process is periodic, by appropriate choice of the saw-tooth voltage frequency, the diagram of the time process appears to be stationary.

A characteristic date of the cathode-ray tube is the sensitivity, which is the displacement of the electron beam on the screen relative to unit deflecting voltage; its order of magnitude is usually mm/V. If the sensitivity is known, or after comparison with a known signal the cathode-ray tube may be used to *measure* signal voltages, signal amplitudes, time intervals, and so on.

(b) To display a two-dimensional image, saw-tooth voltages are usually applied to both plate pairs X and Y (for another method of deflection, see section 5.4.2). The cathode-ray builds up the image from nearly horizontal rows of elements. The horizontal motion is controlled by one of the saw-tooth voltages connected to the deflecting system X, while the vertical displacement of the rows is carried out by another deflecting voltage in the Y direction. The frequency of this latter voltage de-

termines the image frequency, whereas the frequency of the horizontal deflection will be as many times larger than the image frequency as the number of horizontal point rows from which it is desired to produce the image. This is demonstrated in Fig. 5.21, which also contains actual data. The image frequency ensures an image without flashing, and the motion appears to be continuous in the case of an image changing in time. The intensity of the cathode-ray is not constant in this case; it changes from spot to spot, and from moment to moment and the elements of the displayed image differ from each other in brightness. These two-dimensional images are frequently called *B* images (*B* for brightness) especially in ultrasound diagnostics (cf. section

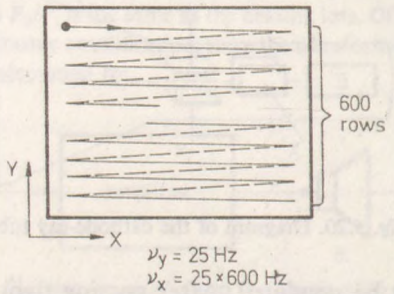

Fig. 5.21. Scanning motion of a cathode-ray

5.4.2). Of the numerous applications of the two-dimensional display tube, we wish to mention here only the scanning electron microscope (cf. section 3.2.3), in which the deflection of the cathode-ray scanning the object and that of the cathode-ray of the image tube are controlled by the same saw-tooth wave generators.

A special type of cathode-ray tube is the memory or storage display tube. This contains an electronic image-storing unit, from which an already displayed image can be recalled to the luminescent screen for prolonged observation or photographing.

Applying computers or microprocessors (cf. section 7.4) the image elements can be stored in the memory. In such cases the storage time can be arbitrarily long and on the display besides the line-diagrams and two-dimensional images, letters, numbers and other symbols (alphanumeric characters) can be displayed, too.

Instead of a cathode-ray tube sometimes a liquid crystal (cf. section 1.4.4) display is applied. Its advantage is the thickness of not more than a few mm (the length of the cathode-ray tube is several hundred mm), its disadvantage is the still small display surface (a few thousands mm^2) and the slowness. This latter disadvantage may be compensated by the very high data acquisition and processing velocity of the used computer or microprocessor system. Liquid crystal displays are used at present mainly for alphanumeric characters.

2. Recorders. It is quite frequently not sufficient to display the signal; it must also be recorded in some lasting form for further evaluation and comparison.

(a) The recorders graphically recording the signals of time processes are called analogue recorders, because of the similarity between the change of the signal in time and its diagram. From the aspect of their application the most imporant character of these recorders is the dependence of the sensitivity upon the frequency of the signal to be recorded. The sensitivity (in the case of cathode-ray tubes the quotient of the deflection and the voltage causing this deflection) can be increased by amplification. They are usually characterized not by the sensitivity of the recorder alone, but by the sensitivity increased by amplification. The magnitude of the sensitivity in recorders constructed for given purposes is a preset value, e.g. in electrocardiographs it is 10 mm/mV. In signal-processing systems with transducers the recorder deflection is usually related to the magnitude of the original signal. Thus, in the recording of arterial pressure, for instance, the sensitivity unit is mm/kPa.

The frequency-independent sensitivity range of recorders (without amplification) begins at zero Hz (direct voltage) and goes up to a frequency depending upon the construction of the device. The high-frequency limit is usually given as a characteristic value, where the sensitivity decreases to seven-tenths of its original value within the transfer band (this sensitivity decrease is equal to an attenuation of 3 dB; cf. point 5 of section 5.3.1). The lower limit of the frequency characteristic of analogue recording systems is given by the *RC* couplings in the units preceding the recorder, while the upper one by the mechanical inertia of the recorder itself. The high-frequency limit of recorders used in medical devices does not usually exceed 100–200 Hz. To characterize the operation of recorders, it should be mentioned that the maximum value of the acceleration of an instrument part recording a sinusoidal signal with an amplitude of 2.5 cm and a frequency of 100 Hz is 10^4 m/s^2. In some special constructions (for instance, in the case of recording with a high-pressure ink beam) the mass (or moment of inertia) of the moving part is so small that the limiting frequency may be one order of magnitude higher. It should be noted that the cathode-ray tube display (and the photographing of the screen image) in the frequency range of medical interest is free of the frequency limit problems.

(b) The practically most simple method of fixing two-dimensional images is to photograph the screen of the cathode-ray tube. However, two-dimensional images can also be obtained with line printers, e.g. in recording scintigrams. In the computerized signal-processing systems the positional coordinates and the data relating to the tones of the pixels of the image matrix are stored in the memory of the computer (e.g. in a magnetic memory). Subsequently, two methods of recording are known. One method is the photographing of the image recalled onto the screen of the cathode-ray tube. In the other method the mosaic printer displays the image composed of matrix elements on the recording paper. Gamma-camera, positron-scanner and computerized X-ray tomography images are made by this method for instance.

5.3.3. Electronic energy sources

Functional units transforming part of the input electric power into electric power with parameters required for special purpose are frequently found in various electronic devices, when for instance the transformation of direct current into alternating current is desired, or vice versa. Similarly, it may also be necessary to transform a lower voltage into a higher one, etc.

1. The sine-wave oscillator produces a voltage varying sinusoidally ($U=U_0 \sin 2\pi\nu t$). This may be achieved simply with an amplifier having positive feedback, whose output is loaded with an *LC* circuit, as depicted in Fig. 5.22. (The feed-back is ensured by an induction coil.) The oscillation begins when the condition $K_F K_P=1$

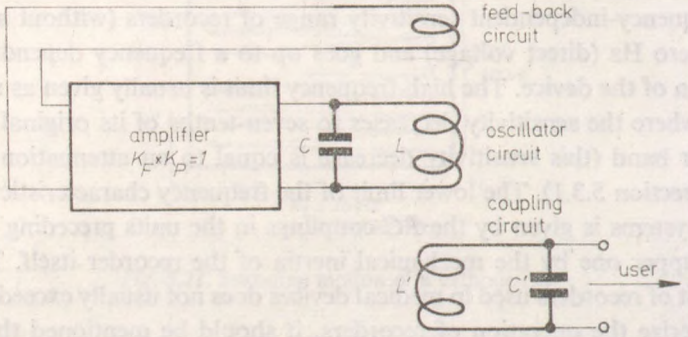

Fig. 5.22. Diagram relating to the sine oscillator

is satisfied (cf. section 5.3.1). The frequency of the electromagnetic oscillation produced is equal to the resonance frequency of the *LC* circuit (ν_0; cf. section 5.2.1), i.e.

$$\nu_0 = \frac{1}{2\pi\sqrt{LC}}$$

ν_0 may be changed according to purpose by a suitable change of C and/or L. The electric energy produced is transmitted to its site of application by an $L'C'$ coupling circuit. The optimum energy coupling can be ensured by the resonance condition

$$LC = L'C'$$

The frequencies used in audiometry are in the range of audible sound (20 Hz to 20 kHz), while those in ultrasonic are higher than 20 kHz. A common feature of all these methods is the transformation of electric power of appropriate frequency into mechanical vibration power. On the other hand, high-frequency heat therapy and surgery utilize radiofrequency range electric power ($\nu > 10^5$ Hz) without any transformation.

2. Pulse generators produce voltage or current pulses and pulse series of a given polarity. The shapes of the pulses (their time course) may differ. In some cases (for

instance in determination of the stimulus characteristics) the shape of the pulse is important, but in other cases (e.g. in ventricular defibrillation) it is of minor importance. Figure 5.23 depicts some characteristic pulse shapes; of these, the simplest and most frequently used is the *square-wave pulse*. Its characteristics are the pulse duration time (τ), the period (T), the frequency ($\nu = 1/T$) and the amplitude (a). Pulse generators involve special electronic circuits. In the following, only square-wave generators are dealt with.

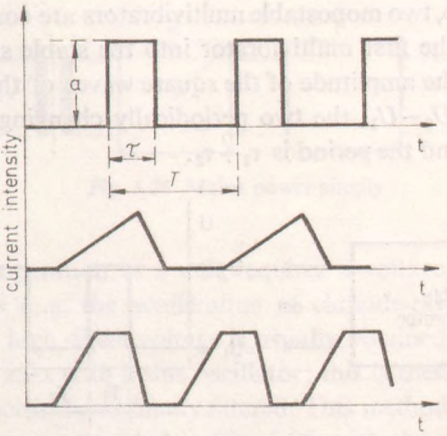

Fig. 5.23. Current pulses of various shapes

Individual square pulses can be generated by a *monostable multivibrator, monoflop*. Besides the stable state, this generator also has an activated (quasi-stable) state, which is produced by a suitable voltage (voltage pulse) applied to the input. The life-time of this activated state depends upon an RC circuit, and it can therefore be expressed by the time constant $\tau = RC$. After this time the stable state is restored. The output voltage has two values: U_1 in the stable state, and U_2 in the activated state (Fig. 5.24). The monostable multivibrator responds to every activating pulse with a square-wave

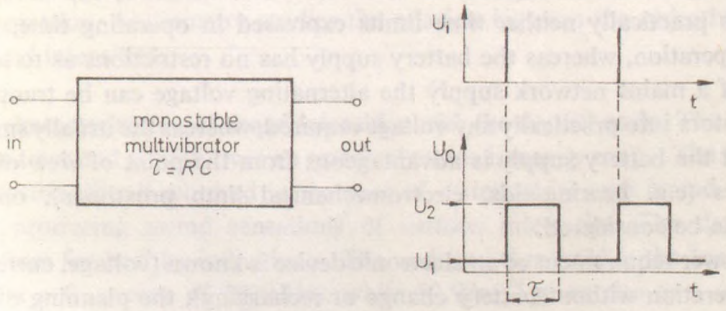

Fig. 5.24. Diagram relating to a monostable multivibrator

pulse, the duration of which is determined by the time constant RC, and whose amplitude is equal to the voltage difference U_2-U_1. This means that this functional unit may also operate as a pulse-shaping device. This property is made use of in ratemeters (cf. section 5.2.1).

If a periodic signal source is added to the monostable multivibrator, a device is obtained whose output releases a series of square-wave pulses. The period is identical with the period of the generating signals. This new functional unit is called an *astable multivibrator,* which naturally operates as a square-wave generator (Fig. 5.25). As the simplest procedure, two monostable multivibrators are connected with each other so that the return of the first multivibrator into the stable state activates the other one, and vice versa. The amplitude of the square waves of the astable multivibrator in this case is again U_2-U_1, the two periodically changing pulse durations are τ_1 and τ_2, respectively, and the period is $\tau_1+\tau_2$.

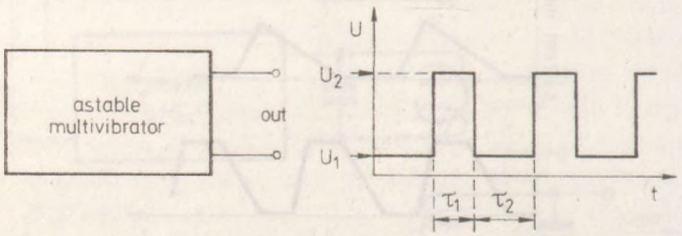

Fig. 5.25. Diagram relating to an astable multivibrator

The direct applications of pulse generators will be dealt with in section 5.5. Here an indirect application in connection with the use of sine-wave generators (for instance in ultrasound diagnostics, or high-frequency surgery) will be discussed. In these cases the power produced in sine-wave generators is used intermittently; this is pulse mode operation. The special switching circuit (gate circuit) regulating the energy output is controlled by a pulse generator.

3. The energy supply of the electronic devices can be obtained in two ways: either from the electric mains network or from a built-in disposable or rechargeable battery. Each solution has advantages over the other. For instance, the mains network supply has practically neither time limits expressed in operating time, nor power limits of operation, whereas the battery supply has no restrictions as to location. In the case of a mains network supply the alternating voltage can be transformed by transformators into practically any voltage required, whereas the usually small voltage means that the battery supply is advantageous from the point of view of safety. In some cases (e.g. hearing-aids, electromechanical limb prosthesis), only battery supplies can be considered.

If the power requirement of an electronic device is known (voltage, current, power, time of operation without battery change or recharging), the planning of a built-in power supply is essentially reduced to the choice between a disposable or rechargeable

battery, and often means a compromise between the operating time and the portability. In the case of a pacemaker introduced under the skin of the patient for instance, a considerable part of the volume and mass of the device is due to its battery, but this ensures operation over several years.

The electronic devices usually require a direct current power supply. Consequently, if an alternating current supplies the power, the required direct power is obtained with a unit consisting of a transformer T, a rectifier R and a filter circuit F (Fig. 5.26).

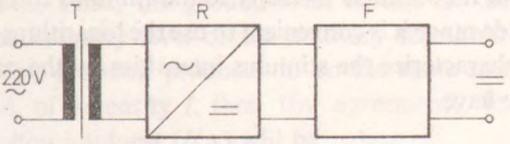

Fig. 5.26. Mains power supply

In some cases the operation of a unit requires a voltage of several hundred or several thousand volts (e.g. the acceleration of cathode-rays). Both in mains and battery operation, this high direct voltage is usually obtained by producing an alternating power of a few kHz with a sine oscillator; this is then transformed to the required high voltage, rectified and finally filtered. This method is similar in essence to the operation of the system depicted in Fig. 5.26, with the single modification that the primary coil of the transformer is supplied by a sine oscillator.

5.4. Applications of sine-wave generators

5.4.1. The physical basis of audiometry

The strength of a sound stimulus is characterized by the intensity of the sound waves, called the sound intensity (objective sound intensity). This must not be confused with the loudness (the subjective sound intensity) characterizing the intensity of *sound sensation*. Audiometry studies the relation between the objective and subjective sound intensities.

1. The characterization of sound intensities with the decibel scale. The human ear is receptive to sound stimuli over an extremely wide intensity range. The table below summarizes the stimuli required in the case of a sinusoidal pure sound of 1000 Hz frequency producing sound sensations of various intensities. The data refer to average values for healthy individuals. The auditory threshold is the lowest audible intensity (at a frequency of 1000 Hz), while 10 Wm^{-2} gives rise to a sensation of pain:

auditory threshold	10^{-12}	Wm^{-2}	shouting	10^{-4}	Wm^{-2}
whisper	10^{-10}	Wm^{-2}	machine room noise	10^{-3}	Wm^{-2}
low-tone conversation	10^{-8}	Wm^{-2}	aeroplane engine noise (at close		
normal conversation	10^{-7}	Wm^{-2}	distance)	1	Wm^{-2}
urban street noise	10^{-5}	Wm^{-2}	pain threshold	10	Wm^{-2}

In practice usually the concept of relative stimulus intensities is used; this is defined as the ratio of the stimulus intensity to the stimulus threshold intensity. With regard to the very wide range it is convenient to use the logarithms of the relative values, or more exactly to characterize the stimulus intensities by the corresponding decibel values (n). Thus, we have

$$n = 10 \log \frac{I}{I_0} \text{ dB} \qquad [5.17]$$

where I is the intensity level of the sound studied and I_0 is that of the stimulus threshold. In the present case $I_0 = 10^{-12}\ Wm^{-2}$. According to the table the intensity level at 1000 Hz corresponding to a low-tone conversation is 40 dB higher, loud shouting is 80 dB higher, and the intensity level corresponding to the pain threshold is 130 dB higher than the stimulus threshold level. The overall intensity range which may be referred to as sound stimulus at 1000 Hz is characterized by a scale ranging from zero dB to 130 dB.

2. The phon scale. The human ear is sensitive to various degrees to pure sounds of various frequencies. Figure 5.27 provides some data on this. The lowest curve depicts the frequency-dependence of the auditory threshold. The other curves demonstrate for various frequencies the intensity values which produce sound sensa-

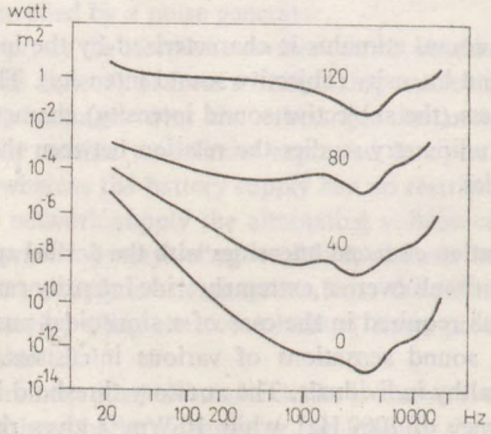

Fig. 5.27. Equal loudness curves of the ear

tions corresponding to a low-toned conversation, shouting or an unpleasant engine noise. (The meaning of the numbers written beside the curves will be given later.)

Similarly as in the case of 1000 Hz, decibel scales may be prepared for other frequencies too. It can be seen from Fig. 5.27 that such scales differ from one another, since different frequency-dependent decibel values are associated with the same sensation. The task is obviously the characterization of the sound sensation independently of the pitch and of the tone. The phon scale was prepared for this purpose.

Let us prepare a decibel scale which measures the intensity level I from the ground level $I_0 = 10^{-12}\,\mathrm{Wm}^{-2}$. Thus, the ground level of this decibel scale corresponds approximately to the intensity level of the auditory threshold of 1000 Hz sound. If, *judging by hearing*, a sound produces in us the same sound sensation as the 1000 Hz pure sound of intensity I, then (by agreement), independently of the *pitch* and *tone*, the phon loudness (H_{ph}) will be

$$H_{ph} = 10 \log \frac{I}{I_0} \text{ phon} \qquad [5.18]$$

The agreement mentioned above solves the problem posed by the frequency-dependent sensitivity of the human ear. Thus, the phon number giving the loudness of a pure sound of 1000 Hz is the same as the decibel value of its intensity level. However, this is not valid for other frequencies. For every sound the auditory threshold is zero phon, while the pain threshold amounts to 130 phon. It may also be said that Fig. 5.27 depicts curves of equal phon loudness for a pure sound. The numbers adjacent to the curves denote the loudness on the phon scale.

In given cases the phon loudness is determined by producing with an audio generator a 1000 Hz sound, whose sound intensity can be varied until the same sound sensation is perceived as for the studied sound. Since the investigated sound and the sound produced by the audiogenerator produce the same sound sensation, the loudness of the studied sound is characterized by the same phon number as that of the sound of 1000 Hz frequency.

The table below gives information about the approximative phon loudnesses of sound sensations of various intensities:

auditory threshold	0 phon	shouting	80 phon
whisper	20 phon	machine room noise	90 phon
low-tone conversation	40 phon	aeroplane engine noise	
normal conversation	50 phon	(at close distance)	120 phon
urban street noise	70 phon	pain threshold	130 phon

3. The sone scale. The previously discussed phon scale is based on the *Weber–Fechner psychophysical law* considered valid for more than a century. According to this law the intensity of sensation is proportional to the *logarithm* of the relative intensity of the stimulus; this is expressed by [5.18] too. In the past decades the investiga-

tions carried out on the relation of stimuli and sensation disproved the validity of the Weber–Fechner law and it was replaced by the *Stevens psychophysical law* corresponding better to experience. According to this law the intensity of sensation is proportional to the *fractional power* of the relative intensity of the stimulus. More exactly, for sound of 1000 Hz frequency at the intensities above 10^{-8} Wm^{-2} (i.e. above 40 dB or 40 phon) — important in everyday practice — the *loudness level in sones* (H_s) can be calculated with sufficient accuracy by the relation

$$H_s = \left(\frac{I}{I_0}\right)^{0.3} \qquad [5.19]$$

Here I_0 is 10^{-8} Wm^{-2} and I is the intensity of the sound in question. The loudness values in phons and sones correspond to each other with a good approximation in the following way:

phon	30	40	50	60	70	80	90	100
sone	0.5	1	2	4	8	16	32	64

Thus it holds for a broad loudness range (at least approximately) that if the phon number increases by 10, the sone number doubles. This relation applies for the whole *sound-frequency range* above 40 phon–1 sone, but cannot be used below 30 phon–0.5 sone. However, this range of low loudness has only slight practical significance.

4. Harmful effects of noise. Intensive or prolonged sound causes not only indisposition and disturbs human activity, but depending on conditions – on top of various nervous and somatic symptoms – may produce temporary or prolonged loss of hearing and in consequence of the irreversible injuries of the internal ear even permanent impairment of hearing. The sources of unpleasant or even harmful noises are mainly the vehicles in traffic and certain industrial and agricultural machines, but more and more noise source can be found among the machines used in housekeeping and the kitchen, and even the electroacoustic devices serving originally for entertainment are well-known noise sources. The purpose of various labour-safety and environment-protection regulations aim to prevent or moderate health impairment and to protect our surrounding against noise.

The various recommendations and regulations allow e.g. for intellectual work a noise level of 35–50 dB, in noisy factories the allowed upper limit is 90 dB, but at about 100 dB already a fast temporary loss of hearing may occur.

Obviously, the harmful effects of noise and the protection against it are not restricted to audible sounds but extend to the non-audible low-frequency infrasound range and to the ultrasound range as well. People working with certain tools (e.g. pneumatic hammer, chain saw) suffer beside the considerable audible noise exposure grave infrasound (vibrational) damage as well.

Regulations concerning the latter fields are still less worked out and in the various frequency bands different parameters are used in the formulation of limits, e.g. the amplitude of vibrations or the ensuing acceleration, in the ultrasound range the power density. Finally it should be emphasized that the *duration* of the stimulus plays an essential role in each range.

5. **Audiometer.** The data summarized above are averages relating to healthy persons. Deviations have been observed for various individuals and various states of health. The sensitivity of the human ear changes with age, the sensitivity (mainly for higher sounds) decreasing in older individuals. The auditory threshold is higher in case of hearing loss. When the sound sensation begins only at 40 phon, for example, the hearing loss of the patient is said to be of 40 phon. The degree of decrease in the hearing in the various frequency ranges depends upon the nature of the illness. A diagnosis is facilitated if the degree of decrease is known throughout the total frequency range. The curve of the decrease in hearing as a function of frequency is an *audiogram*, and the device taking the audiogram is the *audiometer*. As concerns its construction, the audiometer is a sine oscillator whose output is connected with a transducer, for instance an earphone, which transforms the electric signals into mechanical vibrations. The oscillator frequencies can be varied in the frequency range 20–20,000 Hz, and the intensity can be varied at each frequency. The intensity at the measuring frequency must be increased until the individual indicates sound perception. The deviation from the normal auditory threshold can be read directly in decibel (dB) units on the intensity scale.

5.4.2. Ultrasound

1. The ultrasound generator. This device basically consists of a sine oscillator and a transducer. The sine oscillator generates high-frequency (more than 20 kHz) electric power, while the transducer unit converts the electric oscillations into mechanical ones.

The electroacoustic transducers used in the ultrasound range operate on the basis of various phenomena.

(a) *Piezoelectric ultrasound generation.* If pressure is applied to the surface of appropriately cut plates or discs of certain monocrystals (e.g. quartz, ethylene diamine tartrate, Rochelle salt), electric charges are generated. This is the *piezoelectric effect*. The effect is reversible: if electrodes are placed on the crystal plate and a potential difference is applied to the electrodes, the plate will be deformed by the electric field (inverse piezoelectric effect). In an alternating electric field the size (thickness) of the crystalline plate follows the variation of the electric field, i.e. the crystalline plate vibrates. Resonance occurs whenever the frequency of the alternating voltage agrees with the eigenfrequency of the plate. In order to obtain an intensive oscillation, the plate is cut to dimensions according to the frequency of the ultrasound, and the plate is excited by electric oscillations corresponding to the eigenfrequency.

(b) In response to an electric field, a phenomenon similar to the direct or inverse piezoelectric effect occurs in some polycrystalline insulators. This phenomenon is called *electrostriction*, which can similarly be used to generate ultrasound. Discs or

plates of various shapes made of polycrystalline barium titanate are used in ultrasound generators. Piezoelectric and electrostriction transducers may be used to produce ultrasound or to retransform ultrasound into electric signals.

2. The propagation of ultrasound. The velocity of propagation depends upon the frequency to only a small degree, and consequently ultrasound propagates with the same velocity as audible sound in the various substances. Sound is reflected at the boundary of media of different acoustic impedance (i.e. the product of the density and velocity of propagation). For normal incidence the reflectivity (R; cf. section 2.3.1, point 3) can be calculated via the relation

$$R = \left(\frac{\varrho_1 v_1 - \varrho_2 v_2}{\varrho_1 v_1 + \varrho_2 v_2} \right)^2 \qquad [5.20]$$

where ϱ_1 and ϱ_2 are the densities of the two media, and v_1 and v_2 the velocities of propagation in these media. For liquids and solid media the acoustic impedance is generally considerably larger than for gases, and for this reason $R \approx 1$ at liquid–gas and solid–gas boundaries, i.e. most of the sound energy is reflected. Sound energy can be transmitted between solid bodies in air by placing some medium of nearly identical density, a coupling medium between them. If, for instance, ultrasound is to be transmitted into the tissues, the air layer between the irradiating head and the body should be filled with water or oil. Sound propagates with various velocities in various media, and consequently will be refracted when passing boundary surfaces. The short wavelength of ultrasound permits its radiation to be directed and concentrated by lenses and mirrors, etc., of sizes which can be handled easily. As a result of absorption (see below) and scattering, sound intensity decreases. For a parallel beam this decrease can be described by an exponential function. The attenuation is generally larger at shorter wavelengths, and hence ultrasound dies away faster than audible sound. At 10 kHz, for instance, the half-value thickness for air is approximately 100 m, while that for water is approximately 100 km. At 1 MHz the corresponding values are approx. 1 cm for air, a few meters for water, 2 cm for muscle and only a few mm for bone.

3. The effects of ultrasound. In media irradiated with ultrasound complex processes take place.

(a) *Heat effect.* Whenever ultrasound is passed through a medium, part of the vibration energy is transformed into heat. Further, at the boundary surfaces of different media local heating too may occur. The reason for this effect is that the amplitudes of vibration are different in the adjacent media, which undergo friction on each other as it were. This explains the fact that thermometers kept in an ultrasound field show a higher temperature than that in the surroundings.

(b) *Cavitation.* The varying compressive and tensile stresses produced in liquids by ultrasound may overcome the cohesive forces keeping the molecules together, so

that cavities of microscopic dimensions are created. The lifetime of these cavities is short ($<5\,\mu s$) and they soon collapse; energy is released not only as heat, but also in the form of molecular excitation, ionization and dissociation. The development of cavitation requires relatively high intensities. For any given medium the threshold intensity depends upon the frequency, for higher frequencies the threshold intensity also being higher. Absorbed gases make cavity development easier. As an example, it may be mentioned that for mains tapwater the threshold intensity at 800 kHz is approximately 1 W/cm^2.

(c) *Dispersing (emulsifying) and coagulating effect.* Particles suspended in a medium irradiated with ultrasound (in water, air, etc.) vibrate more or less together with the medium. The vibration amplitude of a particle depends on the frequency and intensity of the ultrasound, the dimensions, shape and density of the particles, and the viscosity of the medium. For instance, the amplitude of vibration of a spherical oil particle in water approximates the more closely to the ultrasound amplitude in water, the smaller the radius of the oil drop. Large drops are practically at rest. With increasing frequency, the size of co-oscillating particles decreases.

Particles moving with various amplitudes rub on each other and also on the medium; this results in the breaking-up of the particles, and in a finer distribution (dispersing, emulsifying effect). However, the reverse process may also occur, when strongly vibrating smaller particles collide with large particles which are practically at rest. In this case the small colliding particles adhere to the large ones (coagulation). These two effects usually occur together if particles of various dimensions are present in a suspension. However, the parameters of the ultrasound field can be selected so that either of the two processes predominates. Thus, ultrasound can be used to produce colloid solutions, or very finely distributed emulsions. Further, aerosols may be made with the use of ultrasound, substances with large molecules can be depolymerized, and so on. At the same time, ultrasound is frequently applied in the precipitation of suspensions, the coagulation of aerosols (soot or dust cleaning), the purification of gases from various chemical substances, etc.

In connection with dispersion it should be mentioned that in liquids, besides the above mechanism, an important role is also played by cavitation, sometimes to such an extent that effective dispersion is achieved only in the range of cavitation.

(d) *Chemical effect.* As a result of ultrasound irradiation, similarly to high-energy corpuscular or electromagnetic radiation, in aqueous solutions the water is activated following excitation or ionization of the molecules accompanied by cavitation. The presence of active species (e.g. OH, OH^-, H, H^+, H_2O_2, etc.) in the solution is indicated by oxidation processes. Thus, iodine separates from a potassium iodide solution on ultrasound irradiation.

(e) *Biological effect.* Bacteria, viruses, fungi, smaller invertebrates and vertebrates may be killed by ultrasound. The effect is rather a complex one, as all the mechanical, cavitational, chemical and heat effects of ultrasound must be taken into account. The sensitivities of cells, and human and animal tissues to the destructive effects of

ultrasound differ widely; even a given cell or tissue type displays different sensitivities, depending upon the circumstances of irradiation. Red blood cells, for instance, undergo haemolysis *in vitro* on irradiation with low intensity; *in vivo*, however, even high intensities do not damage these cells. For bacteria, the destructive effect depends strongly upon the culture medium in which the microorganisms are irradiated.

With higher intensities (1–5 W/cm²) the destructive effects of ultrasound predominate, whereas lower intensities (0.2–1 W/cm²) may strongly enhance the metabolic turnover of the same unicellular and multicellular organisms *in vivo*. Besides the effects already mentioned, these phenomena can be explained by the diffusion and cell permeability-increasing effects of ultrasound. Correct ultrasound therapy must be carried out with low intensities, to make proper use of its favourable effects.

4. Some medical applications of ultrasound. For therapeutic purposes (e.g. in case of rheumatic complaints) ultrasound with a frequency of 0.8–1.2 MHz and an intensity of maximum a few W/cm² is usually applied. The therapeutic effect is mainly due to the mechanical pulsating effect (micromassage) and heat production.

The background of the *diagnostic application of ultrasound* is its reflection from the boundary media of different acoustic impedance, and the fact that the time interval between the emission of the ultrasound and the return of its echo is proportional to the distance of the reflecting surface. This permits non-invasive insight into the structure of the tissues. The average ultrasound intensities required for the examination are of the order of magnitude of 10 mW/cm², the physiological effect of which can be neglected.

The essence of this method is demonstrated in Fig. 5.28. Only one point need be stressed. The echo time is represented by a uniform displacement of the cathode-ray in the X-direction. From this it follows that the distance (l) to be determined can be measured by the displacement (l') of the ray as observed on the oscilloscope screen.

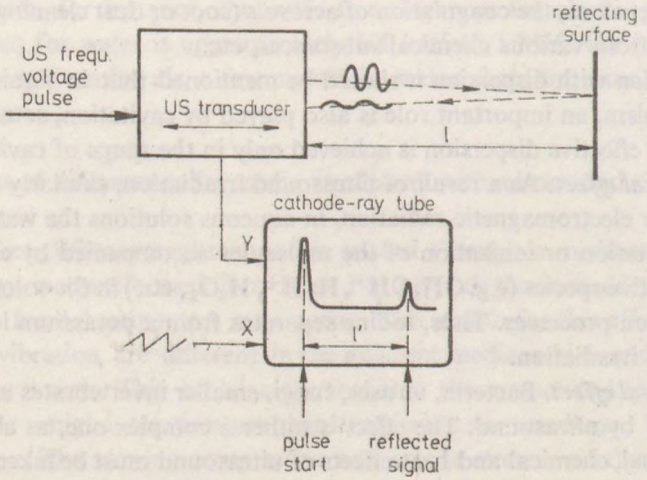

Fig. 5.28. Diagram relating to distance measurement with ultrasound echography

(a) *The examination of static structures.* The echo signals arrive in sequence with a time delay from the acoustically different tissues lying behind one another in the direction of the propagating ultrasound pulse. The distance between the pulses on the oscilloscope screen are proportional to the measured distances of the reflecting surfaces from each other. Further, the amplitudes of the pulses appearing on the screen are determined by the amplitude of the echo pulse. This type of image is an amplitude-modulated or *A* image. The schematic diagram of an *A*-mode ultrasound diagnostic apparatus is presented in Fig. 5.29. With its square-wave pulses of, for example, 1 kHz frequency and 1 μs time period, the pulse generator starts the operation of the saw-tooth wave generator and at the same time opens the gate circuit. From the sine oscillator signals of several MHz frequency in each ms the gate circuit passes an ultrasound frequency signal lasting for 1 μs (several periods) to the ultrasound transducer, which irradiates the ultrasound pulse into the body. The echo signals arrive back in the pauses between the individual input signals and are retransformed into electric signals by the transducer. These signals are then forwarded by the gate circuit (which operates as a receiver in the pulse pauses) into the amplifier. The amplified signals are led to the deflecting system *Y* of the cathode-ray tube and an *A* image is produced.

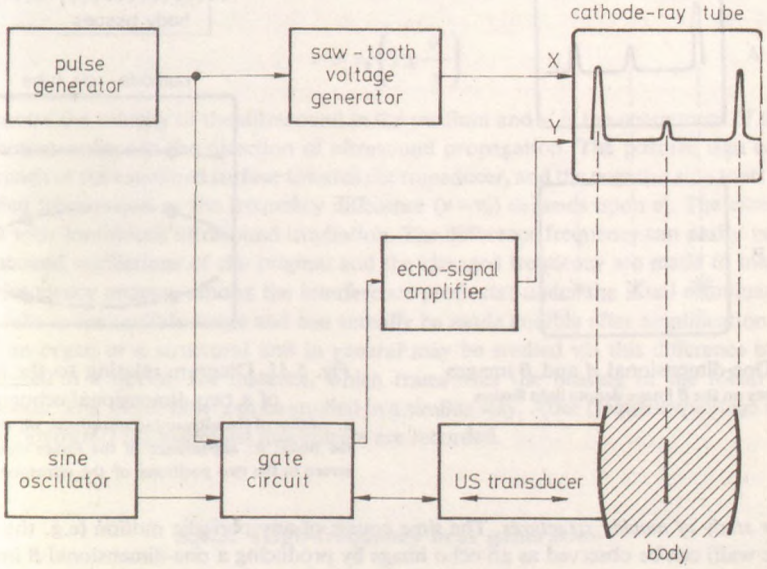

Fig. 5.29. Diagram of ultrasound diagnostic equipment working in the *A*-mode

It is also possible to modulate the current intensity of the cathode-ray with the echo signals. In this case the screen is dark without an echo, and produces a light flash whenever an echo pulse arrives. The image thus obtained is the *B* image (*B*-type operation, cf. section 5.3.2). Figure 5.30 compares one-dimensional *A* and *B* images produced by a standing transducer.

Two-dimensional images may be made from a body section with the aid of a storage tube (cf. section 5.3.2). For this purpose the transducer slides slowly over the skin surface in the plane to be imaged (Fig. 5.31). The motion of the transducer, i.e. its momentary position and the direction of its axis, is transferred to the deflecting system of the cathode-ray tube by a suitable mechano-electric transducer (positional potentiometer). The trace of the cathode-ray spot then moves on the screen in the same way as the ultrasound pulse moves in the body. Thus, the two-dimensional *B* image consists of a set of one-dimensional *B* images drawn in consecutive steps. The brightness modulation

can be solved in two ways. In one case the current intensity of the cathode-ray assumes only two values (zero without echo, and maximum with echo). This gives a bistable image, which consists of dark and bright light spots. In the other technique the current intensity of the cathode-ray is proportional to the intensity of the echo signal. The resulting gray-scale image obviously yields more information (cf. Supplement, Fig. 5.32).

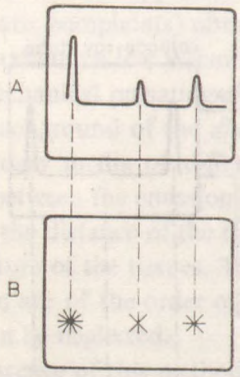

Fig. 5.30. One-dimensional *A* and *B* images
The crosses on the *B* image denote light flashes

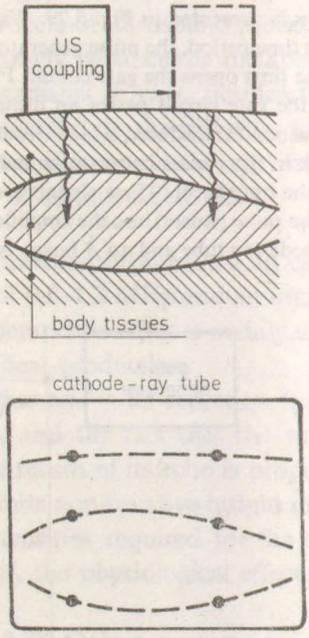

Fig. 5.31. Diagram relating to the production of a two-dimensional echogram
a: motion of the ultrasound transducer on the surface of the body; *b:* appearance of the image elements on the screen in the two positions of the ultrasound transducer

(b) *The study of moving structures.* The time course of any periodic motion (e.g. the motion of the cardiac wall) can be observed as an echo image by producing a one-dimensional *B* image of the examined organ with the aid of a fixed transducer. This type of image is demonstrated by the point series in the *Y* direction on the left-hand side of Fig. 5.33. Consider a single point *P* of the image, which should belong to the outer surface of the heart. If the cathode-ray is deflected in the *X* direction with a rather slow saw-tooth voltage, the change in the distance of the selected point in time will be drawn on the screen. In our example the distance of the selected heart surface point from the transducer (skin surface) will be displayed on the screen. This type of image (which visualizes a motion in time) is called a *TM* (time motion), or briefly a *T* image (*T*-mode of operation).

A two-dimensional moving image of any selected section of a moving structure may also be obtained on the screen. For this purpose, motion (scanning) of the transducer at appropriate velocity and frequency is required. The image appearing simultaneously with the motion of the examined organ is flash-free if the frequency employed is 20–30 images per second. Scanning at required velocity is achieved with a motor drive. However, ultrasound diagnostic equipment exists where the scanning is performed with a static system of transducers. The system may consist of 100 small transducers operating individually in a linear array.

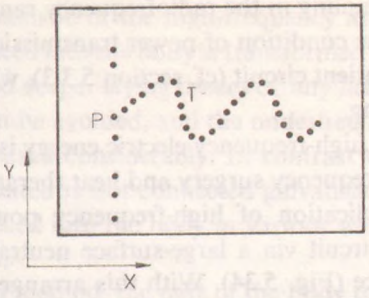

Fig. 5.33. Diagram relating to the formation of a *T* image

In ultrasound examinations of moving structures (the heart, the foetal heart, flowing blood), use is frequently made of the Doppler effect. The basis of this method is the fact that the frequency ν of the ultrasound reflected from a moving surface is different from the original frequency ν_0:

$$\nu = \nu_0 \left(1 \pm \frac{v'}{v}\right) \qquad [5.21$$

where v denotes the velocity of the ultrasound in the medium and v' is the component of the velocity of the reflecting surface in the direction of ultrasound propagation. The positive sign corresponds to the approach of the examined surface towards the transducer, and the negative sign to its departure. The modified frequency ν or the frequency difference $(\nu - \nu_0)$ depends upon v'. The examination is carried out with continuous ultrasound irradiation. The difference frequency can easily be observed if the ultrasound oscillations of the original and the changed frequency are made to interfere. The difference frequency appears among the interference products; under the usual examination conditions, this falls in the audible range and can actually be made audible after amplification. Thus, the motion of an organ or a structural unit in general may be studied via this difference sound. This solution is used in a device, for instance, which transforms the beating of the foetal heart into audible sounds. The blood flow can be studied in a similar way. After frequency/voltage conversion (e.g. with a ratemeter) the examined movements are recorded.

5.4.3. High-frequency heat generation

The heating effect of an electric current does not depend on the current direction or, in the case of alternating current, on the frequency either. Electric power can be transformed into heat as required in the body tissues if the frequency of the current is high enough for its passage not to be accompanied by an excitation effect. This condition is found to be satisfied if currents with a frequency higher than 10^5 Hz are applied. The energy produced in the oscillator of high-frequency medical heat-producing devices is transferred to the site of treatment by the patient circuit (cf. section 5.2.1). The oscillator receives its power from a direct voltage supply or a mains transformer. To avoid electric shocks, the oscillator supply voltage must not pass into the patient circuit. For this reason, an inductive coupling with air insulation is usually used. Thus, the coupling circuit is a high-pass filter circuit (cf. section 5.2.1) which

gives an effective power coupling in the radiofrequency range, and a total separation at the mains frequency. The condition of power transmission is the resonance of the oscillator circuit and the patient circuit (cf. section 5.3.3), which can be achieved with automatic or manual tuning.

The heat obtained from high-frequency electric energy is mainly used in two fields in medical practice: high-frequency surgery and heat therapy.

(a) In the surgical application of high-frequency power the body tissues are connected to the patient circuit via a large-surface neutral electrode and a cutting electrode with small surface (Fig. 5.34). With this arrangement the current relating to unit cross-section, i.e. the current density, will be high close to the cutting electrode. Since the heat produced in unit volume of tissue is proportional to the square of the current density, the warming-up will be stronger at the cutting electrode. The cutting (tissue-separating) effect results from the intense heating under the cutting electrode causing the cells virtually to explode. An important feature of this method is the coagulation due to the heat production, and there is thus relatively little bleeding.

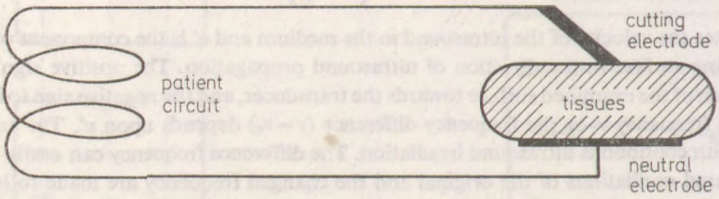

Fig. 5.34. Patient circuit applied in high-frequency surgery

The frequency applied is 10^5–10^6 Hz; with equipment used in major surgery the power is several hundred watts, while in equipment for minor interventions it is lower by one order of magnitude. The current shape is sinusoidal, with a constant or modulated amplitude (Fig. 5.35). In the former case the cutting, and in the latter case the coagulating effect is dominant.

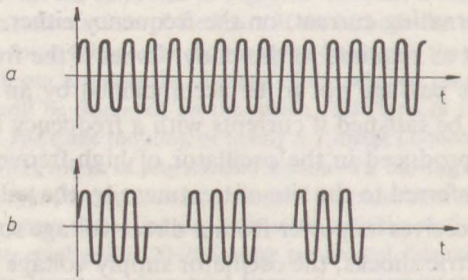

Fig. 5.35. Examples of the high-frequency current forms used in surgery
a: continuous, *b:* pulse modulated shape

(b) A common characteristic of the high-frequency *heat-therapy* methods is that the electric energy introduced into the body is transformed into heat within the tissues. Thus, the introduction into deeper laying tissues of any heat necessary for therapeutic purposes from outside can be avoided, and the undesired heating of the intermediate tissue layers can be decreased considerably. In contrast to the surgical method the part of the body to be treated is not connected galvanically into the patient circuit. The power can be introduced into the body in various ways, associated with characteristic heating conditions.

With the *capacitor field* method, the part of the body to be treated must be placed between the insulator-coated plates of the capacitor of the patient circuit; in the case of the *coil field* method, the part to be treated must be brought beside or inside the coil. In both methods the frequency is several times 10 MHz, and the power a few hundred watts. The body tissues treated in the capacitor field are warmed up as dissipative dielectrics (cf. capacitive current in section 5.2.1). The electric field produced in the individual tissue layers is determined in a rather complex way by all the electric properties (electric conductivity and dielectric coefficient at the applied frequency) of all the layer together. From the aspect of treatment an important result is that the electric field is the lowest in the well-conducting muscle tissues, whereas the heating of the skin and fat tissues is almost ten times that of the muscles. In the treatment with an inductive coil, the intensity of the induced current is proportional to the conductivity of the medium, and accordingly this latter method is favourable from the viewpoint of muscle heating.

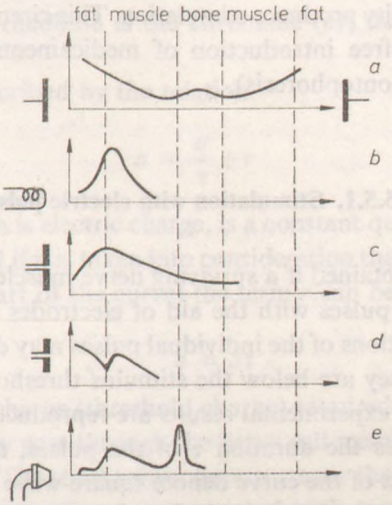

Fig. 5.36. Temperature distributions in the individual tissue layers for various high-frequency heat treatments
a: capacitor field; *b:* coil field; *c* and *d:* microwave field; *e:* ultrasound treatment. The horizontal axis shows the position of the tissues between the capacitor plates and their distance from the coil, i.e. the radiation sources

One of the *radiation field methods* applies a frequency of approximately 2.5 GHz whereas the other operates at 0.5 GHz; the respective wavelengths are approximately 10 and 70 cm. Microwave or decimeter wavelength electromagnetic radiation is directed with a radiator consisting of a half-wavelength dipole antenna and a reflector to the part of the body to be treated. At the frequencies applied, the attenuation coefficient of the fat tissues is approximately five times smaller than that of the muscle tissues, which results in favourable heating effects.

Figure 5.36 compares the various high-frequency (and ultrasound) treatments. The diagram demonstrates the warming-up of various tissues treated together.

5.5. Applications of electric pulses

Several diagnostic methods are based on the characteristic electric phenomena associated with the functions of the cells and organs (cf. section 6.2), which permits the study of these functions. Further, these processes can also be influenced by electric stimulation; this may be used not only for research, but also for diagnostic and therapeutic purposes. The effect of the electric current on a given tissue or cell depends upon the intensity of the current (or more exactly upon the current density), its direction, type, etc. In the following sections this will be illustrated by several applications; only one example is mentioned here. Direct current flowing through the human body remains below the stimulus threshold if the current density is slowly increased up to a current density of approximately 50–200 $\mu A/cm^2$. A rapid increase or a higher current density produces stimulation. This circumstance gives the possibility for the stimulation-free introduction of medicaments electrolytically into the tissues below the skin (iontophoresis).

5.5.1. Stimulation with electric pulses

Experiences can be obtained if a surviving nerve muscle preparation is stimulated by square-wave current pulses with the aid of electrodes applied to the nerve. The amplitudes and the durations of the individual pulses may differ. It is found that some pulses are ineffective (they are below the stimulus threshold), while others result in muscle contraction. The experimental results are reproduced in Fig. 5.37 (full curve). The horizontal axis gives the duration τ of the pulses, and the vertical axis their amplitude (a). The points of the curve denote square-wave pulses which just produce contraction. In other words: the curve depicts the lowest limit of stimulation; this is called the *stimulus threshold* or *stimulus characteristic*. Every point above the curve represents a vs. τ pairs for which stimulation is produced; the points below the curve, on the other hand, relate to a vs. τ pairs for which there is no stimulation. One char-

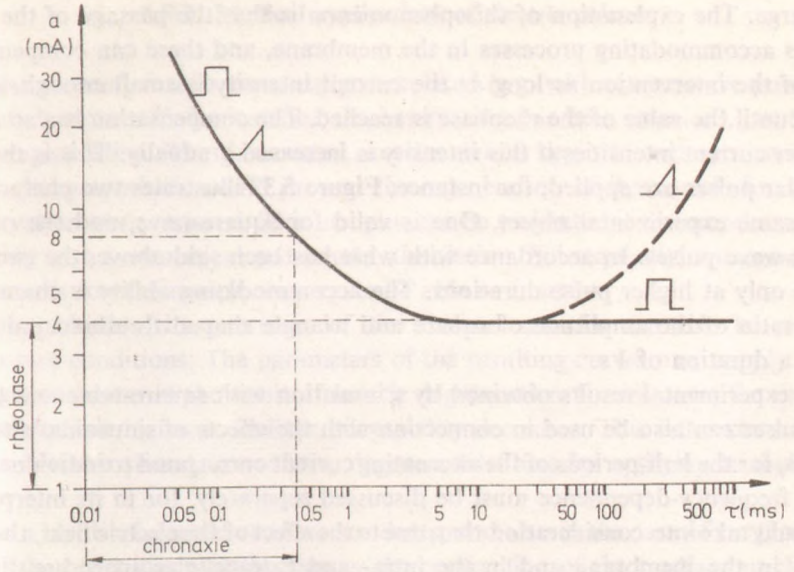

Fig. 5.37. Stimulus characteristic for square-wave and triangle-wave pulses

acteristic datum of the excitability of a nerve muscle preparation is the lowest pulse amplitude which just produces muscle contraction in a sufficiently long time. In the case of the diagram under discussion, the magnitude of this threshold, the *rheobase* (r), is about 4 mA. In the case of a larger pulse amplitude, a shorter pulse duration is associated with the stimulus threshold. The threshold pulse width associated with the double value of the rheobase is the chronaxie (c); its value in our example is approximately 0.4 ms.

The curve can be described by the relation

$$a = \frac{q}{\tau} + r$$

where q, whose dimension is electric charge, is a constant quantity. Its physical meaning can easily be obtained if it is taken into consideration that in the case of sufficiently short pulses (the steep part of the curve) the term r can be neglected relative to q/τ, so that

$$q = a\tau$$

Thus q is the minimum charge (threshold charge) required for stimulation response. The threshold charge may pass through the nerve cell membrane in pulses of varying amplitude and duration. The strong warming-up means that very short pulses cannot be used. Longer pulses are of course associated with a smaller amplitude, but (as already mentioned) pulses with current intensities lower than the rheobase are ineffective. In the horizontal part of the curve, q/τ can be neglected relative to a, which means that the stimulus threshold is now given by the current intensity and not by

the charge. The explanation of this phenomenon is that the passage of the current initiates accommodating processes in the membrane, and these can compensate the effect of the intervention as long as the current intensity is small enough, or more exactly until the value of the rheobase is reached. The compensation is also effective at higher current intensities if this intensity is increased gradually. This is the case if triangular pulses are applied, for instance, Figure 5.37 illustrates two characteristics of the same experimental object. One is valid for square-wave, and the other for triangle-wave pulses. In accordance with what has been said above, the two curves diverge only at higher pulse durations. The accommodating ability is characterized by the ratio of the amplitude of square and triangle shaped threshold pulses both having a duration of 1 s.

The experimental results obtained by stimulation with square-wave and triangle-wave pulses can also be used in connection with the effects of sinusoidal alternating currents, for the half-periods of the alternating current correspond to individual pulses.

The frequency-dependence must be discussed separately, for in its interpretation one should take into consideration that, due to the effect of the electric field, the charge carriers in the membrane and in the intra- and extracellular space are displaced. The motion may be of various types: the translation of ions, the rotation of dipole molecules (atomic groups) and the migration of charge within atoms and molecules. In generating excitation, however, most probably only the translations of the ions (and possibly the rotation of the dipoles) are of importance. However, as a consequence of the relatively large masses of the ions and dipole molecules, these motions are appreciable only if the frequency is not too high. The rapid field-intensity changes accompanying very high frequencies cannot be followed by the "inert" ions (and molecules). The charge motions within atoms and molecules, however, are associated with the oscillations of electrons (of low mass), and consequently these oscillations also occur at high frequencies. These factors do not play a role in the stimulation effects, though they do feature in the production of the heating effect. In the case of higher-frequency alternating current (above 10^5 Hz) a current with an intensity of several amperes may pass through the human body without any stimulation, practically only the heating effect being observed.

With decreasing frequency, the duration of the half-periods (the unidirectional charge motion) increases; the stimulus threshold appears and gradually becomes lower as the frequency decreases. Below a frequency of several 10 Hz the threshold current intensity increases again, as a result of the compensation mechanism already mentioned. It is unfavourable that the stimulus threshold is the lowest near 50–60 Hz, a region of importance due to technical progress (electric hazards, cf. section 5.5.3).

5.5.2. Medical applications of electric pulses

(a) The *skeletal muscles* are usually stimulated (e.g. with square-wave pulses) for therapeutic purposes if, for instance, the innervation has been impaired, but there is hope of regeneration. The denervated muscles would begin to undergo irreversible atrophic changes, which would prevent the regeneration of the muscle functions if the innervation were restored. This atrophy can be prevented if the muscles are kept functioning with systematical and steady stimulation. Subsequently, on regeneration of the innervation the muscle may again be able to function.

(b) The stimulus characteristics of the skeletal muscles can also be recorded under *in vivo* conditions. The parameters of the resulting curves may supply important diagnostic data: in the event of muscle degeneration, for instance, the rheobase and the chronaxie increase, and the adaptability decreases. This latter circumstance allows a selective stimulation of the degenerated fibres by applying triangle-wave pulses.

(c) As a result of electric or other accidents, and also in the case of surgical interventions, the heart may stop beating or the contraction of the cardiac muscles may become uncoordinated (fibrillation). In such cases the application of a short electric shock of high energy to the heart might be life-saving, since the heart muscles then contract simultaneously and subsequently relax. This contraction and relaxation is similar to a natural heart cycle and generally creates conditions favourable for the regeneration of the cardiac functions. The pulse generator applied for this purpose is the *defibrillator*. The defibrillating pulse may be supplied by a capacitor with a capacity of a few 10 μF, charged by a high voltage of several kV. The discharge occurs across the chest within a few ms, in the form of a pulse with an energy of several 100 J.

(d) The pacemaker supplies 70–90 square-wave pulses per minute, thereby permitting regulation of the cardiac function in case of necessity. Implanted pacemakers work quite reliably for several years, supplying ms pulses of a few volts, with an energy of 20 μJ.

5.5.3. Electric hazards and electric safety measures

The hazards of electric current are associated with the stimulating and heat effects of the current. Among the electric power supplies used in everyday life, the electric mains network is of outstanding importance due to its frequent use and inherent dangers. As concerns the sources of danger, we repeat that the stimulus threshold is the lowest around the widely-used frequency of 50 Hz. The danger is increased by the fact that one wire of the mains network is at the earth potential, and thus touching only the other wire may be dangerous if there is no satisfactory insulation between the body and the ground.

The effect produced by an electric current depends mainly upon the intensity, duration and the *path of the current*. This latter expression refers to the organs through which the current flows and the current density developing in them. An especially critical organ from this aspect is the heart, and within it the sinoatrial node.

Table 5.1 presents data giving the approximate lowest limits of the various effects if the current flows between the two arms for longer than 0.3 s. The current intensity follows Ohm's law, and consequently its actual value is determined by the resistance of the circuit at a given voltage. Under the most unfavourable conditions the only

Table 5.1

The effects of electric current*

Alternating current 50 Hz	Direct current	Effect on human body
Current intensity mA		
1—1.5	5—6	weak shock sensation (sensation threshold)
15	70—80	beginning of danger (painful spasm in limb muscles); "let go" *current intensity*
25	90—100	respiratory spasm, heavy pain
80	300	ventricular fibrillation, danger of death
100	500	cardiac paralysis, clinical death

* Data of the International Labour Organization (1961)

appreciable resistance in the circuit is the human body resistance, in which the skin surfaces play a decisive role. The resistance of dry skin is greater than of moist skin, and thus the resistance of the body depends primarily on the degree of wetness of the skin. Figure 5.38 gives information on the resistance of the human body between two hands of intact skin at various voltages. It may be calculated from the data in the diagram that even 50 V may have serious consequences under unfavourable conditions.

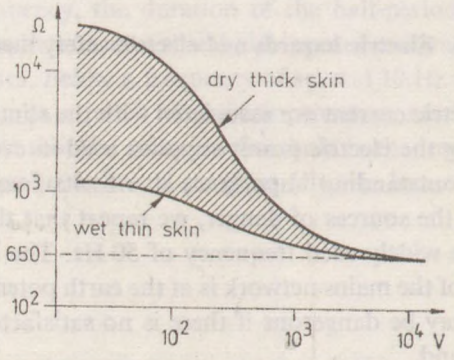

Fig. 5.38. Informatory data on the resistance of the human body

At lower voltages (a few hundred volts), or at moderate current intensities, the contractive symptoms are dominant. The higher current intensity due to a higher voltage produces a larger current density on the heart, similarly to the effect of a defibrillating pulse. In a "lucky" case the dangerous consequences due to contraction do not appear, and thus at higher voltages the burning symptoms predominate.

The data of Table 5.1 relate to cases when the current enters and leaves on the body surface (macroshock). However, if the entrance and exit occur on the cardiac surface, e.g. when a catheter is applied (microshock), it is found that a current intensity of even a few 10 μA may cause fibrillation. The generally accepted safety threshold for microshocks is thus 10 μA.

It follows from the above that the use of electric power, i.e. the application of electric/electronic devices, may be dangerous and represents some risk. The possibility of electric accidents can be decreased by means of various shock-proof technical devices and by observing the relevant safety regulations. These were developed on the basis of experience and the actual technical solutions and regulations usually differ depending on the type of the device and the circumstances of its application. It is generally true that the regulations are more rigorous for medical devices than for equipment used in everyday life (for instance in the household), for with certain medical devices *good electric contact* must be made between the patient and the instrument via the electrodes placed on the patient's body. This, of course, increases the risk. It is extremely important to know and observe the electric safety regulations in every field of application of medical devices.

5.6. Signal processing

Electronic diagnostic devices are generally signal-processing systems. As already mentioned (section 5.1), the signal is related to some process or event (sequence or groups of events) occurring in the system. Accordingly, with some simplification, we may refer to the processing of continuous signals and of pulse signals.

5.6.1. Processing of continuous signals

1. The measuring system is shown schematically in Fig. 5.39; the signal source is the patient. The coupling element (cf. section 5.2.1) ensures a selective connection, specific for the signal to be processed, between the signal source and the processing system. Originally, in the case of an electric signal (e.g. an action potential associated with the muscle function) the coupling elements are simply contacts (electrodes), but in other cases the coupling element transforms the signal to an electric one (transducer). The amplifier amplifies the signal to the higher level required for further processing. The transfer characteristics of the amplifiers must be adjusted to

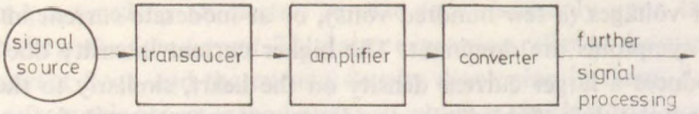

Fig. 5.39. Block diagram relating to the processing of continuous signals

the signal to be processed. The frequencies of the biologically or medically important signals are in the range 0–10 kHz; however, in practice a narrower frequency band within this range is generally used. Direct voltage (0 Hz) is required for measurement of the body temperature or in concentration measurements using electrochemical transducers. In the recording of cardiac sounds the amplifier should amplify signals in the frequency range 10–800 Hz.

The further processing of signal may mean e.g. the elimination of noise signals or the frequency-analysis of the signal (e.g. its filtering; cf. section 5.2.1.9); however, signal processing with computers (microprocessors) is getting more and more general (cf. sections 5.6.4 and 7.4.1). In respiratory function analysis e.g. the detector produces a voltage signal proportional to the intensity of expiration and after processing the recorder gives not only the intensity versus time curve, but numerous other diagnostic data too, as e.g. the vital capacity or the highest expiratory intensity even expressed in the percentage of normal (healthy) data. In some cases the transformation of electric signals into electric signals with different parameters may be necessary to simplify or even render possible the further processing.

2. Signal conversion. The transformation of an electric signal into an electric signal with different parameters is called *signal conversion,* and the functional unit converting the signal is the *converter*. It is an evident requirement of signal conversion that the information content of the signal must not change during the conversion. Signal conversion is necessary, for example, in cases when a varying signal voltage (e.g. an ECG signal) has to be transmitted by some means to some distance (telemetry; cf. section 5.6.3). It is a well-known fact that in telecommunications the frequency is a much more stable parameter than the amplitude. Thus, the solution of the previous task will be a conversion resulting in an unambiguous (e.g. proportional) assignment of the frequencies of an alternating voltage (or pulse series) to the various values of the signal voltage. This is the case of voltage→frequency conversion. A signal converted in this way is suitable for either telecommunications or magnetic storage (on a magnetic tape or disc). By means of a reversed (frequency→voltage) conversion, the original signal voltage can be reconstructed and, if necessary, recorded. The signal voltage and the signal frequency may be regarded as analogue signal parameters since any change of these signals in time may be analogous to the change of the original signal in time. For this reason these conversions are referred to as *analogue→analogue conversions*. Of the basic circuits (cf. section 5.5.1), the ratemeter is also an analogue–analogue converter.

Modern technology applies digital computers in an ever widening territory (cf. section 7.4.3) for signal processing. This requires an *analogue→digital* conversion of the amplified signal which assigns digital data to the numerical values indicating the instantaneous magnitude of the signal voltage. This sampling should be done with a frequency high enough in order to ensure an unchanged information content of the complex biological signals (cf. section 6.3.2). This is usually attainable if the frequency of sampling is at least double of the highest frequency component of the signal (cf. Table 6.4). In the case of a computed X-ray tomograph (cf. section 2.12) the signals of the transducer detecting the X-ray intensity transmitted by the body section are fed into the computer after an analogue→digital conversion. The computer stores the calculated elementary density data in digital form and consequently the display of the densitogram will be preceded by *digital→analogue* conversion.

Digital computers and calculators operate in a binary system, though the data are fed in and the results are displayed in the decimal system. Both the input and the output involve *digital→digital* conversion (cf. section 7.4.1). The converters may be accessories or interfaces of the computer.

3. Multichannel measuring systems and other constructions. The system depicted in Fig. 5.39 is suitable for the study or recording of only one signal at a time. Several such or similar systems *(channels)* are frequently built together into one piece of equipment. With multichannel equipment several signals can be studied or recorded simultaneously (synchronously). Their best-known applications are the simultaneous multielectrode detecting and processing of various action potentials (cf. section 6.3).

For the recording of action potentials, the measuring equipment is completed with various stimulators; thus, the action potentials of the central nervous system can be stimulated repeatedly with light and sound pulses. The phono- and photostimulators for these purposes are electronically-controlled pulse-operated sound or light sources. Examination of the action potentials of the muscles, or measurement of the velocity of nerve conduction, requires similar equipment, where electric pulses are employed for the reproducible production of action potentials.

In special cases (during operations, deliveries involving complications, etc.) or in critical conditions (e.g. after an operation), measuring systems may be required which are suitable for the display or possibly the recording of the most important body functions and parameters. Thus, bedside monitors most often contain channels to measure or record the electrocardiogram, the pulse and respiratory rate, the temperature, the pulse and the blood pressure. It is a general requirement that intensive care systems give alarm signals whenever any parameter goes outside the preset range.

5.6.2. Processing of pulse signals

Determination of the *frequency of some event* is a much used diagnostic method. Examples of such parameters to be determined are the concentrations of the blood components, and the distribution of radioactive isotopes in the body. The technical solution of the measurement of such signals requires the processing of pulse signals.

A block diagram of the processing system is depicted in Fig. 5.40. The signal source may be the patient if the determination relates to the distribution of a radioactive isotope introduced into the organism, or it may be a blood sample if the number of red blood cells are to be determined. The role of the detector is to assign voltage pulses to the individual events. After appropriate amplification, these pulses are classified (discriminated) according to amplitude. The further processing involves either *counting* or *frequency determination* (cf. ratemeter; section 5.2.1), with subsequent display or recording and computer processing. In the following section a few remarks are made in connection with some functional units.

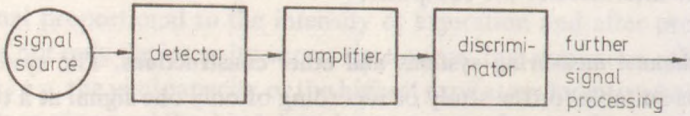

Fig. 5.40. Block diagram relating to the processing of pulse signals

1. Detectors have already been dealt with, mainly in connection with the measurement of nuclear radiation (cf. section 2.15). As an example here, we should like to describe a detector which is used to count the blood components (Fig. 5.41). The blood sample, diluted with physiological salt solution, is pumped from the outer vessel to the inner one through a capillary at the bottom of the inner vessel. Meanwhile, by means of the two electrodes a current of low intensity flows through the system. Whenever a blood element passes the capillary, the resistance of the electrolyte in the capillary increases, which produces a voltage pulse.

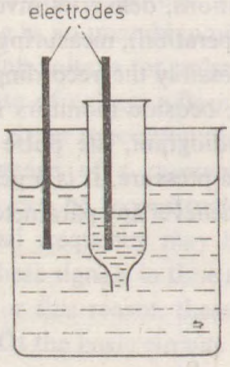

Fig. 5.41. Diagram demonstrating the counting of the blood elements (measuring capillary)

2. Discriminators. The amplitude of the output pulses of the above detector is proportional to the dimensions of the blood elements. A similar statement can be made in connection with the scintillation detector: its output pulses are proportional to the energy transferred to the substance of the scintillator by the gamma-photon. Often the classification of pulses according to amplitude *(pulse-height analysis)* may yield valuable information. For this purpose electronic units, discriminators, are used. These have two modes of operation.

(a) *The integral discriminator* (Fig. 5.42) gives a pulse on its output only when the amplitude of the pulse on the input is larger than the preset discriminating *threshold voltage* ($U_{\text{threshold}}$). The integral discriminator responds to every pulse above the threshold with a uniform output pulse.

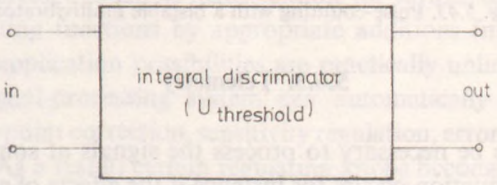

Fig. 5.42. Diagram relating to the integral discriminator

(b) Pulse-height analysis is carried out in the *differential discriminator* operation mode. The difference between the differential and the integral discriminator is that in this mode of operation an upper threshold voltage can be set above the discrimination threshold. A pulse appears on the output whenever the amplitude of the input pulse falls between the two preset limits, i.e. it enters the *discrimination channel.* With the differential discriminator the frequency distribution of the pulse amplitudes, i.e. the pulse amplitude spectrum, can be obtained.

3. Pulse counters. The individual pulses can be counted relatively easily by means of a *bistable multivibrator.* This is a functional unit with two stable states (Fig. 5.43) and two definite values of the output voltage. The change of state (and the change of the output voltage) is triggered by an input voltage of suitable amplitude. The next pulse resets the previous state (together with the corresponding output voltage). Thus the bistable multivibrator responds with one square pulse to the two input pulses. However, this means that the bistable multivibrator halves the pulse number. A pulse-dividing (pulse-counting) chain is obtained if these multivibrators are connected so that the output pulses of one unit are passed to the input of the next one. The counting is carried out in a binary system, the two values of the output voltages of the individual multivibrator units corresponding to the two digits of the binary system. Their place values are given by the individual multivibrator units. The results of the counting are converted into the decimal system with a digital–digital converter (cf. section 7.4.1).

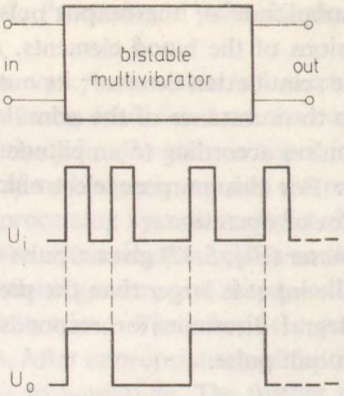

Fig. 5.43. Pulse-counting with a bistable multivibrator

5.6.3. Telemetry

It may sometimes be necessary to process the signals at some distance from the signal source. This situation occurs for instance if the effects of special circumstances or stresses on the physiological parameters are to be examined. A well-known example is the control on the Earth of the vital functions of astronauts in space, but sport physiology and labour hygiene may also acquire valuable information through telemetry. The problem is solved by connecting a telemetric channel between the signal source and the processing system. In more simpler cases this can be done with conducting wires; for instance, the patients in the intensive care units of hospitals are connected to the central observing system by means of wires.

In other cases only wireless communication is possible, because the wires would hinder the activities to be observed. As an example, the telemetric processing of ECG signals may be mentioned: a small UHF radio transmitter is attached to the patient. After amplification, the ECG voltage is converted into sound frequencies (cf. section 5.6.1), the carrier frequency being modulated by these sound waves. The ECG signal is then reconstructed, i.e. processed at the receiver side.

5.6.4. Medical electronics and computers

The use of computers will be discussed later (cf. section 7.4.2); here, only their role in signal processing is mentioned.

Complex and exact evaluation, especially in case of a large number of signals, is possible only with the use of computers. As an example, the analysis of EEG or ECG signals might involve the evaluation of complex signals obtained in 3–250 channels (cf. section 6.3.2). The connection of the signal-supplying equipment to the computer may be direct; another possibility is to feed the computer with stored data (e.g. data stored on magnetic tapes).

Many instruments, for instance large, diagnostic equipments, operate with their own built-in computer. Examples are transmission or emission computed tomographs (cf. section 2.12), gamma-cameras (cf. section 2.18), computerized ECG diagnostic systems and medical expert systems (cf. section 7.4.2). Reference may also be made in this respect to the diagnostic laboratory automatic equipment, which can be used, for example, to move sample holders containing urine samples, to add reagents necessary for colour reactions, to determine concentrations and to store the data systematically. All these processes are operated by computerized systems.

The application of microprocessors in the field of medical equipment initiated the development of special possibilities and solutions. Microprocessors can take over the functions of the central unit of digital computers (cf. section 7.4). They are not computers themselves, but can be made suitable for various calculating, counting, comparing or regulating functions by appropriate additions (memories, input and output units). Their application possibilities are practically unlimited. For instance, a microprocessor signal-processing system can automatically carry out its own standardization, zero-point correction, sensitivity regulation, error display, measuring-channel change, etc. As a result, certain regulating knobs become superfluous, which simplifies the use of the device and makes its operation more reliable. In this way e.g. after putting on the electrodes and starting the ECG apparatus by pushing a single button it records successively the usual 12 ECG curves (meanwhile stabilizes the zero level, checks the skin–electrode resistances and the sensitivity of recording) and finally it prints the data obtained from the computer analysis of the ECG signals on the recorder chart. It is even possible in intensive care systems for parameters of ECG signals (time intervals, amplitude values) to be compared continuously with preprogrammed data or with the data of the previous cardiac cycle and the device signals any unfavourable tendencies in due time.

5.6.5. Imaging systems

Various methods resulting in the production of two-dimensional images are employed in medical diagnostics. The simplest of these are photographs of the outer or inner surface of the human body. Some of these methods are based on up-to-date technical devices such as the introduction of light by light-conducting fibres. This is the technique of fibre optics, which is suitable not only for the introduction of the illuminating light, but also for the production of the image by the aid of an ordered fibre bundle. Electronic devices exist for the same purpose, and in this way photographs and even motion pictures can be taken of the movements of the foetus within the uterus.

X-rays provided the first non-invasive insight into the human body. The traditional X-ray photographs were summation shadow images in which the three-dimensional human organ was reduced to a two-dimensional image. Computed X-ray tomography (cf. section 2.12) depicts the third dimension lost in the formation of the

summation shadow image since the tomograms are images of the section perpendicular to the body axis. The MRI method (cf. section 3.5.1) too supplies a section image when by means of nuclear magnetic resonance the spatial distribution of proton concentration or that of relaxation time can be obtained.

Numerous image-producing methods have been developed to follow the distribution of radioactive isotopes in the body in space and time (cf. section 2.18).

The reflection of ultrasound likewise lends itself to the development of valuable image-producing diagnostic methods (cf. section 5.4.2). It should be borne in mind that, in contrast with the procedures employing ionizing radiation, no risks exist with the ultrasound methods.

Finally, a further non-invasive image-producing method without any risks may be mentioned. The method prepares images of the surface of the body using only the thermal radiation of the body itself (cf. section 2.4). The method depicted in Fig. 5.44 is called thermography. At every moment, a mirror rotating about a vibrating axis reflects a small element of the body surface into the transducer, which is sensitive

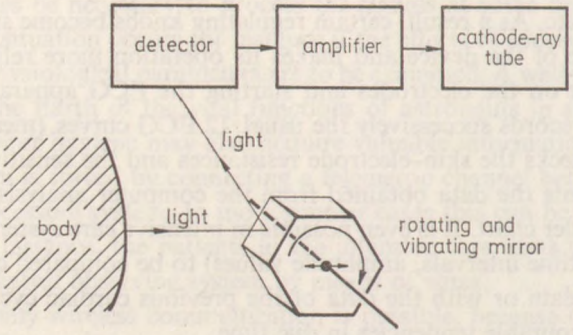

Fig. 5.44. Diagram relating to thermography

to infrared radiation. The fast vibration of the mirror axis scans the surface horizontally, and its slow rotation scans the surface vertically; with the mediation of the mirror, therefore, the body is actually scanned by a transducer in horizontal lines. The momentaneous values of the transducer signal denote the temperatures of the corresponding points on the body surface. The deviation of the cathode-ray is synchronized with the motion of the mirror system. In this way a heat map of the body of the patient is produced on the display.

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6. THE BIOPHYSICS OF EXCITATION PROCESSES. EXAMPLES OF PHYSICAL MODELLING

At any degree of organization a characteristic feature of living cells is the excitability. This is one of the conditions necessary for the living organism to adapt to its environment. In higher organisms excitability is primarily a property of certain specialized cells. Outstanding examples of this are muscle and nerve cells. It is understandable, therefore, that a vast amount of knowledge has accumulated in this field, and various efforts have been made to give a generalized interpretation of this branch of biophysics. This explains our purpose in selecting this field of biology to discuss the interrelation between theory and experience, which is an essential factor in scientific progress.

6.1. Electric properties of resting cells

An understanding of excitation processes requires the knowledge of the properties of resting cells with respect to their electric characteristics. If, for instance, appropriately shaped, non-polarizing electrodes are positioned within a resting muscle cell (in the intracellular space) and on some point of the cell surface (in the extracellular space) (Fig. 6.1), a potential difference, the *resting potential,* can be measured between the electrodes. The intracellular electrode is always found to be at a negative poten-

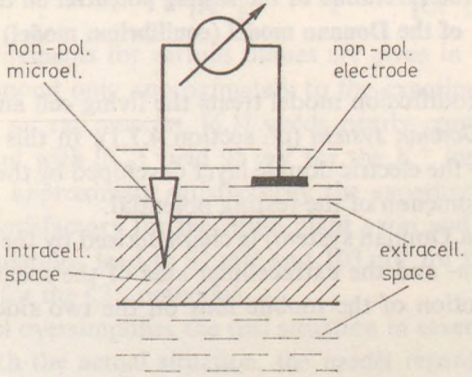

Fig. 6.1. Diagram relating to measurement of the resting potential
The convenient extracellular medium is ensured by a salt-solution

tial with respect to the extracellular electrode. The values of the resting potential differ depending upon the cell type and the animal from which the cell originates. Even with identical cells, this value also depends upon the composition and concentration of the ionic constituents of the solution surrounding the cell. The resting potential corresponding to the normal ion composition in the intra- and extracellular spaces is generally 80–100 mV.[1] In some cases the resting potential is given as a negative quantity, for the potential of the extracellular space is usually taken as zero, when the potential of the intracellular space will be negative. In the following treatment the resting potential is regarded as negative only in the diagrams; the discussion relates to its absolute value.

Several models have been developed to interpret the resting potential (and generally the stimulatory processes). The older though still most frequently used models describe the processes phenomenologically by thermodynamical reasoning, which is still effective and by far not weakened by the more recent developments based on a deeper knowledge of the details of the molecular mechanism. Since the thermodynamic models connect the development of the potential with the diffusion of the ions within the cells and in the intercellular space across the membranes, they are called *electrodiffusion models*. In these theories an attempt is made to explain the movement of the ions across the membrane as well as the blocking of the movement by the characteristic properties of the structural elements of the membrane. Since the *molecular interpretation* considers the lipid double layers and the properties of the proteins connected looser or tighter to the lipids, these models are usually referred to as *the solid-state physical models*. The electric behaviour of the resting cells is frequently described also by *electric circuits*.

The models discussed in this chapter in some detail have been selected according to their success in interpretation, their extent, general acceptance and also expressivity.

6.1.1. Interpretation of the resting potential on the basis of the Donnan model (equilibrium model)

The simplest electrodiffusion model treats the living cell and its intra- and extracellular spaces as a *Donnan system* (cf. section 4.7.1). In this model the membrane potential produced by the electric double layer developed by the presence of immobile ions is the basic phenomenon of the resting potential.

The living cell (as a Donnan system) is characterized by the presence of immobile ions on both the intra- and the extracellular side of the membrane; these together determine the distribution of the mobile ions on the two sides of the system (Fig.

[1] A potential difference of 100 mV between the two sides of a cell membrane approximately 10 nm thick corresponds to a field strength of about 10^7 V/m.

6.2). The protein and phosphate anions of the intracellular space are immobile, and to a first approximation the cell membrane is also impermeable for the Na^+ ions which are found mainly in the extracellular space. From the aspect of the Donnan model, the mobile K^+ and Cl^- ions are the most important ions in the cells and in the intercellular space.

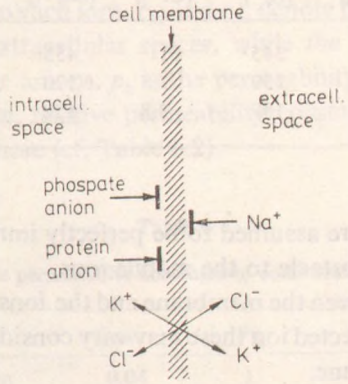

Fig. 6.2. Distribution of mobile and immobile ions in the intra- and extracellular spaces

In this model, because of the presence of the immobile ions, the concentration of K^+ ions ($[K^+]$) is higher in the intracellular space than in the extracellular fluid, whereas for the Cl^- ion concentration ($[Cl^-]$) the situation is the reverse (cf. section 4.7.1):

$$\frac{[K^+]_i}{[K^+]_e} = \frac{[Cl^-]_e}{[Cl^+]_i} \qquad [6.1]$$

where the subscripts i and e refer to the intracellular and the extracellular space. The potential difference ($\varphi_e - \varphi_i$) between the two sides of the membrane may be described by the equation

$$\varphi_e - \varphi_i = \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_e} = \frac{RT}{F} \ln \frac{[Cl^-]_e}{[Cl^-]_i} \qquad [6.2]$$

Some results of measurements for various tissues are given in Table 6.1. The model may be said to correspond only approximately to the experimental results. For the tabulated tissue data on rat muscles, [6.1] yields nearly correct results. Similarly, calculations carried out with [6.2] yield 95 mV for the K^+ ions and 86 mV for the Cl^- ions. These data approximate satisfactorily the experimental value of 92 mV. The situation is less satisfactory for the squid giant axon and the frog muscle. For these tissues the calculations lead to 91 mV and 103 mV for the K^+ potential, and to 56 mV and 89 mV for the Cl^- potential.

The Donnan model oversimplifies the real situation in several respects:

(a) In contrast with the actual situation, the model regards the cell and its environment as a thermodynamically closed system and studies the equilibrium conditions accordingly.

Table 6.1

Measured values of ion concentrations and resting potentials for a few types of tissue

Tissue	Intracellular conc. (mmol/l)			Extracellular conc. (mmol/l)			Resting potential (mV)
	$[Na^+]_i$	$[K^+]_i$	$[Cl^-]_i$	$[Na^+]_e$	$[K^+]_e$	$[Cl^-]_e$	
Squid giant axon	72	345	61	455	10	540	62
Frog muscle	20	139	3.8	120	2.5	120	92
Rat muscle	12	180	3.8	150	4.5	110	92

(b) The immobile ions are assumed to be perfectly immobile, and the membrane is assumed to present no obstacle to the mobile ions.

(c) The interactions between the membrane and the ions are not taken into consideration, though for any selected ion these may vary considerably depending upon the composition of the membrane.

Accordingly the further disadvantageous situation follows that the model considers only one type of mobile ion at a time (e.g. K^+ or Cl^-).

6.1.2. Interpretation of resting potential on the basis of the Hodgkin–Huxley–Katz model (transport model)

The model to be discussed in this section contains fewer simplifications than the Donnan model (cf. section 4.7.2, point 2). The main characteristics of this model can be summarized as follows. A constant concentration difference exists between the outer and inner sides of the membrane, which results in a constant material transport across the membrane. The model is not concerned with the processes maintaining the concentration differences; there are no restrictions in this respect. Ions participate in the transport, their migration across the membrane being hindered to various extents, and hence an electric double layer is produced on the two sides of the membrane. The resting potential is equal to the potential difference characterizing the double layer. One of the advantages of this model is the possibility that all of the ion species on the two sides of the membrane can be considered simultaneously. Further, it also takes into account the empirical fact that the membrane is neither perfectly permeable nor totally impermeable for any ion type. The permeability of the membrane is different for the different ions.

Consequently, it follows that the Hodgkin–Huxley–Katz model is based on an equation describing the ion transport across the membrane (cf. section 4.7.2). For the membrane potential, i.e. the resting potential $(\varphi_e - \varphi_i)$, this equation yields the following relation

$$\varphi_e - \varphi_i = -\frac{RT}{F} \ln \frac{\sum_{k=1}^{n} p_k^+ c_{ke}^+ + \sum_{k=1}^{m} p_k^- c_{ki}^-}{\sum_{k=1}^{n} p_k^+ c_{ki}^+ + \sum_{k=1}^{m} p_k^- c_{ke}^-} \qquad [6.3]$$

Since generally monovalent ions are considered in the development of the resting potential [6.3] refers only to such ions. c_{ki} and c_{ke} denote the concentrations measured in the intracellular and extracellular spaces, while the superscripts "+" and "–" refer to the cations and the anions. p_k is the permeability constant of the membrane for the kth ion. In practice, relative permeability constants are used and in a given case p_k is substituted by these (cf. Table 6.2).

Table 6.2

Relative permeability constants of some resting cells*

Tissue	p_{Na}	p_K	p_{Cl}
Squid giant axon	0.04	1	0.45
Frog muscle	0.01	1	2

* Related to the permeability constant for potassium

With the tabulated data, [6.3] yields 61 mV for the squid giant axon and 90 mV for the frog muscle at 25 °C. The agreement between the measured and calculated values is better than with the Donnan model. Attempts to obtain even better agreement appear superfluous, for the differences are within the error of measurement.

Further *electrodiffusion* models developed to explain the resting potential with the migration of ions across the cell membrane emphasize certain characteristic properties of the molecular mechanism. Special attention is usually given to the interpretation of the membrane permeability for Na^+ and K^+ ions. With appropriate selection of the conditions, the solution of these models generally leads directly or indirectly to [6.3] or some similar relation.

6.1.3. Hyper- and depolarization and their modelling

Basic phenomena. Besides the electrodes detecting the resting potential, let us place another pair of electrodes inside a fibre and on its surface (Fig. 6.3). With the aid of these latter electrodes, electric current pulse (usually square pulses) are passed through the membrane. In this way a transient change of the membrane potential can be produced. The former electrode pair is the measuring, and the latter the exciting electrode pair. If the current direction (the direction of the shift of the positive charges) is from the surface electrode towards the intracellular space, the numerical

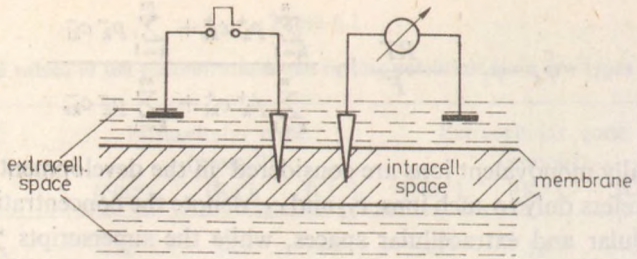

Fig. 6.3. Diagram relating to the change of the resting potential
On the left the exciting electrode pair; on the right the recording electrode pair.
In the case discussed the distance between the two electrode pairs is of the order of a tenth of a mm

value of the membrane potential increases; for the opposite direction, the potential decreases, or may even change in sign. The first case is called *hyperpolarization* (Fig. 6.4*a*), and the second *depolarization* (Fig. 6.4*b–c*). Hyper- and depolarization as depicted in Figs 6.4*a* and *b* can be characterized in a relatively simple way: the maximum change of the voltage observed on the measuring electrode develops later relative to the exciting pulse. The difference between the excitation and response is more marked when the depolarization attains a certain value (Fig. 6.4*c*), since in this case an essentially new phenomenon is produced. A stimulus inducing only a local depolarization is a stimulus *below the threshold,* while a stimulus which by producing a depolarization induces the excitation process of the cell is a *stimulus above the threshold.* (In the example presented in the diagram the stimulus below the threshold amounts to a few tenths of a μA, and it is a square pulse with a duration of a few ms.) In the present section only locally induced hyper- and depolarization are dealt with; the excitation processes of the cell will be discussed in section 6.2.

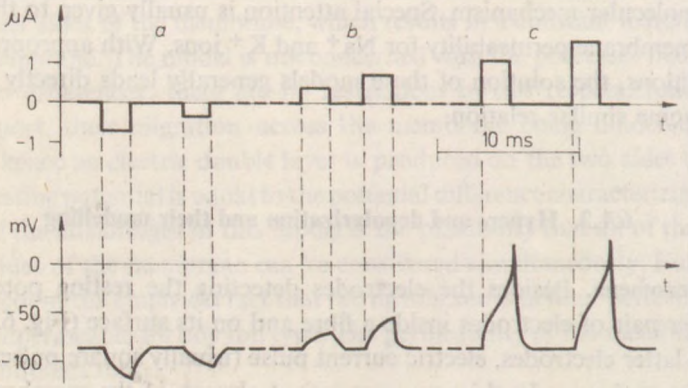

Fig. 6.4. The effect on the membrane potential (lower diagram) of square-wave current pulses connected to the cell membrane (upper diagram)
The upper ordinate shows the amplitude of the current pulses, and the lower one the resting value of the membrane potential (ca. −80 mV) and its changes. The abscissa gives the time

Modelling. The electric properties of the membrane involved in hyper- and depolarization and the excitation processes to be discussed in section 6.2 are modelled with the electric circuit depicted in Fig. 6.5. The drawing presents identical interconnected units. Each individual unit models a definite section of the membrane, of cross-resistance R_m and capacity C_m. The individual units are connected with each other by the longitudinal resistances on the intra- and extracellular sides, R_i and R_e. Every quantity is related to the membrane length. It is worthwhile emphasizing the empirical fact that $R_e \ll R_i$. U is the membrane potential, U_0 is its resting value, and E is the voltage due to the concentration difference of the mobile ions on the two sides of the membrane, as obtained from the Donnan model by the Nernst equation (cf. sections 4.5.3 and 4.7.1). Consequently, using the notations of [6.2], we have

$$U = \varphi_e - \varphi_i;\ E = \frac{RT}{F} \ln \frac{[K^+]_i}{[K^+]_e} = \frac{RT}{F} \ln \frac{[Cl^-]_e}{[Cl^-]_i} \qquad [6.4]$$

U and E act in opposite directions. At rest (in equilibrium): $U_0 = E$.

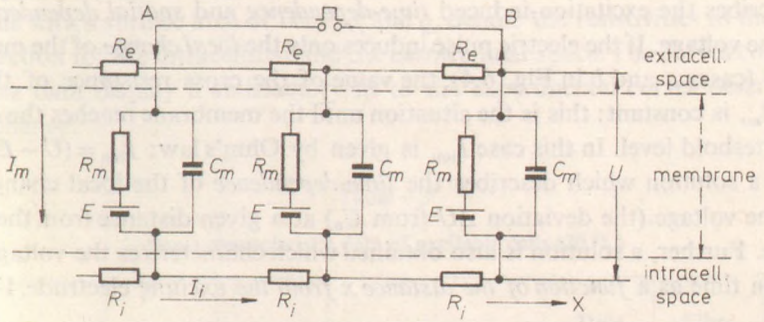

Fig. 6.5. Schematic circuit of the electric model of the cell for the interpretation of the effect of a pulse on the membrane
A B: exciting electrodes

A current pulse induces a current both along and across the membrane. From Fig. 6.5 it is clear that the current intensity (I_m) across the membrane, referred to unit membrane length, can be expressed in two ways. From considerations not given in detail here, we have

$$I_m = \frac{1}{R_i} \frac{d^2U}{dx^2} \qquad [6.5]$$

where d^2U/dx^2 is due to the change of the modified membrane potential along the membrane (X-axis). In the derivation of [6.5] it was taken into consideration that the change of the current intensity I_i along the membrane is equal to the current intensity across the membrane. Since $R_i \gg R_e$, it is sufficient to consider only R_i in the derivation. Another possibility of expressing I_m may be understood easily by

inspecting Fig. 6.5. The electric behaviour of the membrane in the cross direction can be modelled by a parallel *RC* circuit, and consequently I_m consists of two parts. One is the ion migration (I_{ion}) through the resistance R_m, and the other is given by the capacitive current (I_C) on the capacitor. Thus:

$$I_m = I_{ion} + I_C \qquad [6.6]$$

Every symbol denotes the current intensity referred to unit membrane length. From the definition of the capacity, I_C is given by

$$I_C = C_m \frac{dU}{dt} \qquad [6.7]$$

where dU/dt gives the time-dependence of the voltage change on the two sides of the membrane.

Combination of [6.5], [6.6] and [6.7] leads to the differential equation

$$\frac{1}{R_i}\frac{d^2U}{dx^2} = I_{ion} + C_m \frac{dU}{dt} \qquad [6.8]$$

[6.8] describes the excitation-induced *time-dependence* and *spatial dependence* of th membrane voltage. If the electric pulse induces only the *local change* of the membrane-potential (cases *a* and *b* in Fig. 6.4), the value of the cross resistance of the meme brane, R_m, is constant: this is the situation until the membrane reaches the depolarization threshold level. In this case I_{ion} is given by Ohm's law: $I_{ion} = (U-E)/R_m$ and [6.8] has a solution which describes the *time-dependence* of the local change of the membrane voltage (the deviation ΔU from U_0) at a given distance from the exciting electrode. Further, a solution is also obtained which characterizes the voltage change at a given time as a *function of the distance x from the exciting* electrode. Figure 6.6

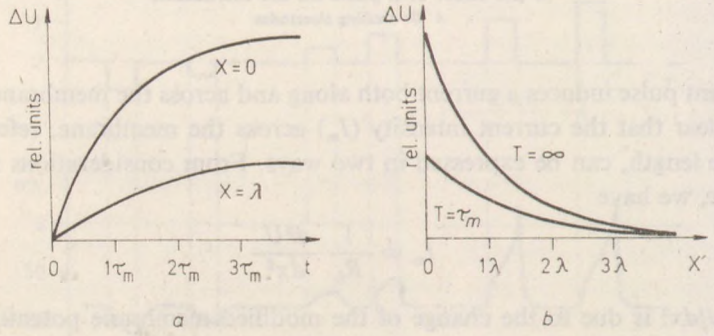

Fig. 6.6. Time course (*a*) and spatial distribution (*b*) of the local membrane potential change. ΔU is the change of the membrane potential with respect to the resting potential value U_0
$X=0$ is the site of excitation, $X=\lambda$ is the membrane length constant distance from the site of excitation; $T=\infty$ relates to the development of the maximum membrane potential change, and $T=\tau_m$ relates to time τ_m after the excitation

presents some solutions of the differential equation [6.8]. Figure 6.6*a* depicts the time-dependent change of the resting potential from the beginning of the exciting pulse ($t=0$) at the position of the exciting electrode ($x=0$), and at a distance λ from it. It should be noted that the characteristics of the curves are similar to those of the voltage change recorded in the time period of the pulse in cases *a* and *b* in Fig. 6.4. For the voltage change after the pulse is stopped, [6.8] yields a theoretical solution similar to the declining branches of the curves in Fig. 6.4. The λ value in Fig. 6.6*a* is the *length constant*, which is the distance at which the pulse-induced voltage change has decreased by a factor e from its initial value. The τ_m value on the abscissa is the *time constant*. The value of the membrane time constant is given by the product $C_m R_m$, while the expression $\sqrt{R_m/R_i}$ gives approximately the value of the *length constant*. Figure 6.6*b* depicts the solution of [6.8] which yields the expected voltage change on moving away from the point of excitation ($x=0$). The curve $T=\infty$ demonstrates the change in the maximum value of the voltage, whereas the other curve shows the situation at the time $T=\tau_m$ after the start of the exciting pulse.

The electric quantities of the model can be determined experimentally. In Table 6.3 the quantities ϱ_m and γ_m are the cross-membrane resistance and capacity of a membrane with a surface area of 1 cm². ϱ_i and ϱ_e denote the resistivities in the longitudinal direction for the intracellular and the extracellular space. The curves constructed with these data display a satisfactory fit to the experimental curves describing the voltage change.

Table 6.3

Some characteristic data of excitable cells (20 °C)

Fibre type	ϱ_i (Ω cm)	ϱ_e (Ω cm)	ϱ_m (Ω cm²)	γ_m (μF/cm²)	Time constant (ms)	Fibre diameter (μm)	Membrane length constant (cm)
Squid axon	30	22	700	1	0.7	500	0.5
Lobster nerve	60	22	2000	1	2	75	0.25
Crab nerve	60	22	5000	1	5	30	0.25
Frog muscle	200	87	4000	6	24	75	0.2

6.2. Electric properties of excited cells

Curves *a*, *b* and *c* of Fig. 6.7 refer to responses to square current pulses for a frog muscle fibre. Curves *a* and *b* correspond to local depolarization, whereas curve *c* represents the response to excitation above the stimulus threshold. This latter phenomenon is related to the *excitation processes of the fibre*.

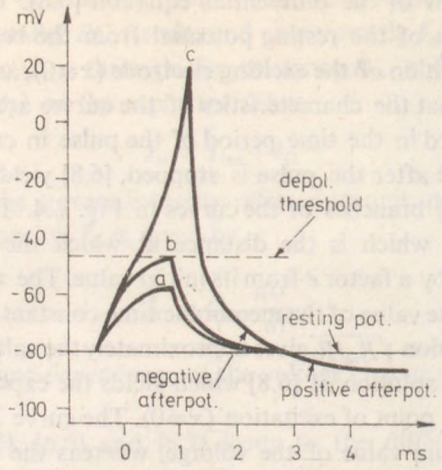

Fig. 6.7. Local depolarization response (curves *a* and *b*) and action potential (curve *c*) due to a square-wave pulse on frog skeletal muscle

6.2.1. Action potential of a single fibre

A change in membrane potential similar to curve *c* of Fig. 6.7 is obtained whenever an excitation process is triggered in a single fibre. This phenomenon is called an *action potential*. It is generally true that, independently of its intensity, each stimulus reaching or exceeding the depolarization threshold level produces identical action potentials.

The action potentials can be characterized by various data, for instance by the maximum voltage change, the *potential peak*, which in the case of a frog muscle fibre extends from -80 mV to $+20$ mV as demonstrated in Fig. 6.7. Thus, the total change in this case amounts to 100 mV. The rising part of the peak is the *depolarization*, and the descending part the *repolarization*. It generally holds that the former of these two processes is the faster. From the duration of the depolarization, which is within 1 ms, and the height of the potential peak, the *depolarization rate* is found to be 10^2–10^3 V/s. Further characteristics are the *duration* of the action potential, and the presence, magnitude and duration of *after-depolarization* and *after-hyperpolarization potentials*. The action potentials of various cell types differ in the height and duration of the peak and in the re- and depolarization processes.

Of the quantities associated with the action potential, the stimulus threshold level should be mentioned; its value (in the diagram ca. 30 mV) is not constant even for a given system: it depends strongly upon the state of the system and, since it changes continuously within the physiological limits, the threshold level fluctuates at about an average value.

The stimulus threshold changes characteristically in the course of the action po-

tential too. Of these changes, only the most striking ones are discussed here. During the period of the potential peak the stimulus threshold becomes infinitely large, which means that a new stimulus cannot induce an excitation process; from the viewpoint of excitability this is the absolute refractory period. After the peak the stimulus threshold is higher for some time than the normal one (the relative refractory period); afterwards it reaches its resting value through a strongly damped oscillation.

6.2.2. Phenomena connected with the action potential and their modelling

The development of the action potential is an extremely complex process. This complexity is due to the different changes of the membrane permeability for various ions, and also to the special modifications of the migration conditions of the individual ions. In the following section the processes will first be described qualitatively, and the possibilities of a quantitative description will subsequently be shown.

1. Action potential–membrane permeability. It is observed that every change of the membrane potential is followed by a change of its permeability. Let us follow this process in the course of the action potential. First, when the depolarization threshold level is exceeded, the permeability of the membrane increases mainly for the Na^+ ions. As a consequence, a large number of Na^+ ions will flow towards the intracellular space in accordance with the concentration gradient. The presence of the Na^+ ions brings the negative potential of the intracellular space nearer to zero, i.e. the depolarization increases. However, this further increases the membrane permeability for the Na^+ ions, and a self-amplifying process is induced *(Hodgkin cycle)*. The process lasts until other effects terminate the depolarization e.g. the migrations of the K^+ and Cl^- ions, which likewise increase during the depolarization. These latter phenomena are somewhat delayed with respect to the increase of the Na^+ flux, and their predominance is indicated by the decrease of the action potential subsequent to the peak. The depolarization-decreasing effect of the K^+ and Cl^- ion fluxes can be understood easily by considering the fact that, corresponding to the concentration gradient, there is a K^+ efflux and a Cl^- influx. Thus, the effects of the two types of ions finally cause the potential of the intracellular space to become more negative. The described mechanism operates until the development of the resting state and even somewhat longer, overshooting it thereby producing an after-hyperpolarization potential (cf. Fig. 6.7). Subsequently the initial position is restored. The occurrence of this process therefore indicates that the system which restores the resting potential operates by a negative feedback process (cf. section 7.2).

2. Quantitative characterization of ionic fluxes. The main question associated with the process of the action potential is how the membrane permeability and the

current vary with time for different ions, and how these values depend upon the *actual value of the membrane potential.* Answers to these questions are given by the empirical results; [6.8] provides a full quantitative description of the action potential. The measurement of the time-dependence of the ionic fluxes is carried out by the *voltage clamp* technique. The essence of the method is to keep the membrane potential at a fixed value during measurement, which can be achieved easily with a suitable regulating circuit.

In the voltage clamp method, besides the measuring electrodes a second electrode pair is used; the voltage setting the actual membrane potential (U) must be applied suddenly to these. If the set voltage reaches or exceeds the depolarization threshold level, an action potential will be induced, and an ion movement characteristic of this will be produced. Of course, this would change the set voltage U. The change is compensated by a voltage applied to the second electrode pair and in this case the compensating current intensity is always equal to the actual ionic flux to be measured. Besides the total ionic flux, the method allows measurement of the individual ionic fluxes. For this purpose some suitable substance must be added to the extracellular fluid, which blocks the membrane permeability for the Na^+ or the K^+ ion (e.g. tetrodotoxin or tetraethylammonium ion).

Figure 6.8*a* presents as an example the time-dependence of the ionic fluxes (or more exactly the current densities) for a single constant membrane voltage, when $U=0$ mV. Figure 6.8*b* depicts the kinetics of the membrane conductivity. This can be calculated from the results of the measurements. The diagram shows that whenever the membrane voltage rises suddenly from its resting value to above the depolarization threshold level (in the present case from -60 mV to 0 mV) the conductivity of

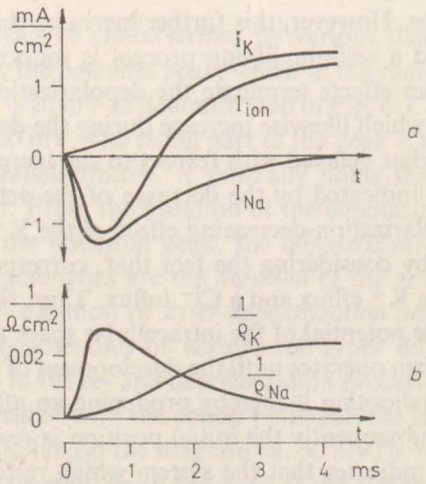

Fig. 6.8. Kinetics of ion current (*a*) and conductivity (*b*) changes produced in response to a voltage change ($\Delta U=60$ mV) applied to the membrane

I_{Na}, I_K and I_{ion} denote the Na^+, K^+ and total ionic current densities. Negative values on the ordinate denote a positive ion influx, and positive values an efflux; conductivity of the membrane for Na^+ and K^+ ions denoted by $1/\varrho_{Na}$ and $1/\varrho_K$, respectively

the cell membrane suddenly increases for the Na^+ ion and returns to the initial value only gradually. For the K^+ ion, on the other hand, the sudden change of the voltage results in a gradual increase of the conductivity. Thus, in accordance with the conductivity change, the resultant flux initially consists mainly of a Na^+ influx, followed subsequently by a K^+ efflux.

The time-dependences of the ionic current densities can also be determined at membrane voltages different from the case shown in Fig. 6.8. If the maximum ionic current density produced (the saturation value) is assigned to each voltage value, Fig. 6.9 is obtained; this depicts the dependences of the two most important ionic current densities on the membrane voltage. It may be seen from the curves that the

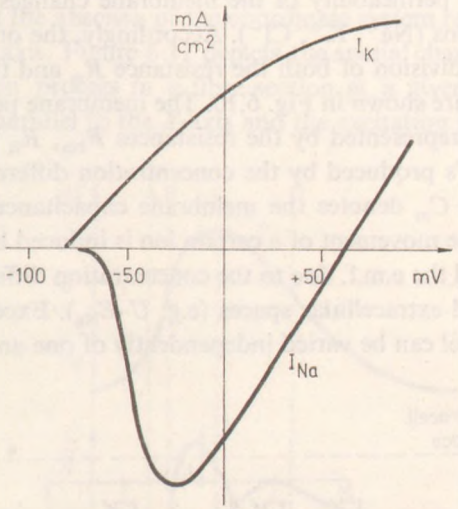

Fig. 6.9. Dependence of the maximum ionic current densities on the membrane voltage, kept at a constant value by the voltage clamp technique

Na^+ and K^+ ions differ considerably from each other in behaviour. It is obvious that there exists a membrane potential at which the movement of the Na^+ ions ceases. This voltage, the *equilibrium potential,* is approximately 60 mV in our case. Its value is equal to that calculated from the intra- and extracellular Na^+ concentrations via the Nernst equation (cf. section 4.5.3). The sign of the equilibrium voltage is opposite to that of the e.m.f. due to the concentration difference of the Na^+ ions. In other words: the equilibrium voltage compensates the e.m.f. originating from the concentration difference of the Na^+ ions, and hence impedes the movement of these ions. It should be mentioned that the equilibrium voltage for Na^+ ions in the case of the rat muscle is 62 mV, for the frog muscle 44 mV and for the squid axon 45 mV. Consequently, if the membrane voltage approaches or reaches the value of the equilibrium potential, the movement of Na^+ ions decreases and finally stops. There-

fore, the equilibrium potential is an important factor in the reversion of the self-amplifying process associated with the movement of the Na^+ ions, and it also plays an important role in promoting the predominance of the processes (the movement of the K^+ ions) leading to the original state, i.e. to the resting membrane potential.

3. The electric model of the action potential. The electric processes discussed in the previous point can be interpreted by the further development of the model considered in section 6.1.3. Let us begin with the circuit presented in Fig. 6.5, which will be amended on the basis of the previous discussion. The *ionic movement* across the membrane associated with the excitation processes can no longer be treated in a uniform way, as was the case with the local hyper- and depolarization, for during the excitation process the permeability of the membrane changes in different ways for the more important ions (Na^+, K^+, Cl^-). Accordingly, the original model circuit is supplemented by the division of both the resistance R_m and the e.m.f. E in the RC circuit. The divisions are shown in Fig. 6.10. The membrane permeabilities for Na^+, K^+ and Cl^- ions are represented by the resistances R_{Na}, R_K and R_{Cl} and E_{Na}, E_K and E_{Cl} are the e.m.f.'s produced by the concentration differences of the respective ions. In this case too C_m denotes the membrane capacitance and U is the actual membrane voltage. The movement of a certain ion is induced by the difference of the membrane voltage and the e.m.f. due to the concentration difference of the given ion between the intra- and extracellular spaces (e.g. $U-E_{Na}$). Experience shows that the resistances in the model can be varied independently of one another.

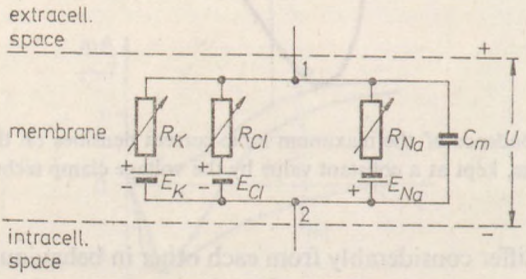

Fig. 6.10. Block diagram of one unit of the electric model suggested by Hodgkin, Huxley and Katz

In an excited cell, similarly as for hyper- and depolarization, a current flows along the fibre in the intra- and extracellular spaces as well as across the membrane. In this case too the membrane current (I_m) consists of the ionic (I_{ion}) and the capacitive current (I_C), which means that [6.8] also holds for excitation. However, in order to characterize the excitation process quantitatively, [6.8] must be supplemented with the empirical results relating to the ionic current densities:

$$I_{ion} = I_{Na}(U-E_{Na},t)+I_K(U-E_K,t) \qquad [6.9]$$

To a first approximation it is sufficient to consider only the Na^+ and K^+ ions. [6.9] expresses the fact that the difference between the actual membrane voltage (U) and the equilibrium potential (E) of the given ion and the time-dependence of the membrane resistances, which are different for the two most important ions (cf. Fig. 6.8*b*) together determine the values of the ionic current densities.

As in the case of the resting potential, [6.8] supplemented with [6.9] has two possible solutions. One characterizes the time-dependence of the action potential, and the other relates to its *propagation* (spatial dependence). In both cases the solutions yield results in agreement with experience. As an example, without giving in detail the solution of the differential equation characterizing the action potential Fig. 6.11 demonstrates graphically the propagation of the excitation. The solution was obtained with the assumption that the rate of propagation along the fibre is constant. This assumption means that the abscissa of the coordinate system representing distance is equivalent to the time axis. Figure 6.11 depicts the spatial changes of the data characterizing the excitation process in a fibre section at a given moment. The nerve fibre in this case runs parallel to the X-axis and the excitation propagates from right

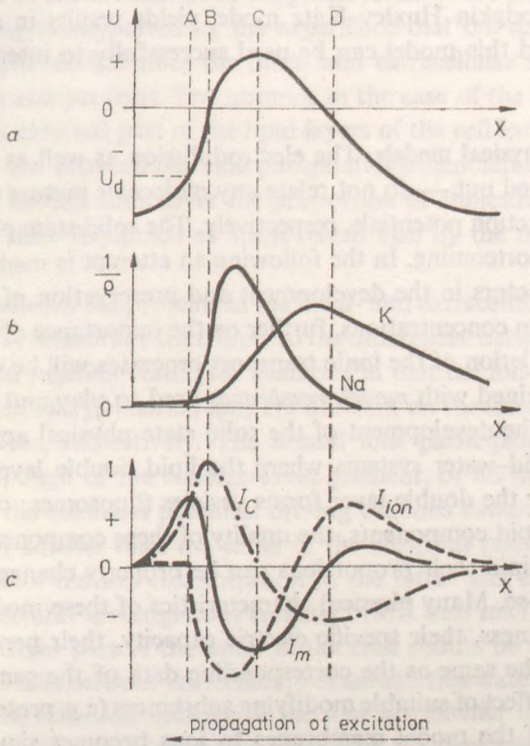

Fig. 6.11. One possible solution of the electric model of the action potential: the spatial distribution (X) of the data characterizing the state of the excited fibre at a given moment

U: actual membrane potential; $1/\varrho$ = specific conductivity; I_m = membrane current; I_C = capacitive current; I_{ion} = ionic current densities related to unit membrane length

to left. The dashed line *C* indicates the plane of maximum excitation in the fibre. To the left of this plane the excitation develops while to the right the repolarization process occurs. Diagram *a* represents the spatial change of the membrane potential. At the moment shown, the action potential peak lies in the plane *C*, while the planes *B* and *D* denote the sites of the steepest depolarization and repolarization changes, respectively. Diagram *b* gives the change of the membrane permeability (conductivity $1/\varrho$) for Na^+ and K^+ ions, and diagram *c* depicts the change of the resultant membrane current density (I_m) and its components (I_{ion} and I_C) in the course of depolarization and repolarization, respectively. As previously, the negative current direction again indicates the influx of the positive charges.

The Figure leads to the following conclusions. An appreciable ion current is observed only from the point (*A*) where the membrane voltage reaches the depolarization threshold level (U_d). Before this site the ion current is very low and a considerable capacitive current can be observed. These results are in agreement with phenomena discussed in section 6.1.3. At the potential peak (*C*) a large ionic influx is observed, which corresponds to the resultant of the maximum Na^+ influx and the simultaneously increasing K^+ efflux. During the repolarization the K^+ efflux predominates. To summarize, the Hodgkin–Huxley–Katz model yields results in agreement with the empirical facts, and this model can be used successfully to interpret the action potential.

4. Solid state physical models. The electrodiffusion as well as the electric models — as already pointed out — do not relate any molecular picture to the development of the resting and action potentials, respectively. The solid state physical model helps to eliminate this shortcoming. In the following an attempt is made to sketch the role of the structural factors in the development and preservation of the different intra- and extracellular ion concentrations, further on the importance of the structure in the operation and regulation of the ionic transport processes will be explored.

The results obtained with *model membranes* used to play and still are playing an important role in the development of the solid state physical approach. The model membranes are lipid–water systems where the lipid double layer is either a single lipid membrane, or the double layer forms vesicles (liposomes; cf. section 1.5.5). In these systems the lipid components, the quality of these components, and in the case of more than one lipid their proportions can be properly changed according to the experimental purpose. Many physical characteristics of these model membranes (for instance their thickness, their specific electric capacity, their permeability to water) are quantitatively the same as the corresponding data of the genuine cellular membranes. Due to the effect of suitable modifying substances (e.g. proteins, oligopeptides), the permeability of the model membranes to ions becomes similar to that of the cellular membranes. The model membranes provide a well-defined experimental system which allows to carry out physical measurements in exact (controlled) condi-

tions and also make possible the (e.g. statistical physical) interpretation of the experimental results on a molecular level.

(a) In the production of the difference in the ion concentrations of the intra- and extracellular spaces an important role is attributed to the active transport, i.e. the migration of the ions against the concentration gradient. The active transport created by metabolic processes at the cost of chemical energy is realized by the protein constituents of the membranes, the so-called *ion pumps*. The molecules participating in the operation of the various pumps are different for various cells and species. Their examination has been carried out up to now only for a few instances.

One sphere of the problems interpreted by the solid state physical models refers to *the structural factors* which contribute to the preservation of the concentration difference. The more important of these factors are the structure of the cell membranes and their constituents, respectively, further on the free or bound state of water and the ions. At present it appears that both factors participate to a certain degree in the development of the phenomenon, though the extent of their participation is still not clear. The model attributes some importance to the different behaviour of the *bonding sites* at the two sides of the membrane in the preservation of the concentration differences. This reasoning is supported by the experience that the structure of the cell membranes is not symmetrical since the intra- and extracellular solutions are built up to different lipids and proteins. For instance in the case of the membranes of the red blood cells at the external part of the lipid layers of the cell lecithin and sphingomyelin, whereas on the intracellular side phosphatidylethanolamine and phosphatidylserine are found. In this latter layer the proportion of cholesterol is smaller than on the extracellular side. Asymmetries are revealed also by the membrane proteins of the red blood cells.

The differences between the proteins in the intra- and extracellular space found on the two surfaces of the membrane contribute to the differences between the two membrane surfaces, which together result, for example, in that the ion-binding capacities concerning the sodium and potassium ions are different on the internal and the external membrane surfaces, respectively. The bound ions participate only to a small degree in the development of the concentration gradient, or do not participate in it at all. Accordingly, the chemical potential driving the ions toward the smaller concentration is actually smaller than the value of the chemical potential as calculated from the mean value of the ion concentration in the intra- and extracellular space.

The result of structural investigations (mainly NMR and microcalorimetry) also demonstrate that a larger part of the *water* in the cells should be present in a ***bound state***, i.e. its structure falls between the structure of ice and free water (cf. section 1.5.1). The solubility and the diffusion velocity of the ions are smaller in the bound water than in free solutions. It should be noted here that the decrease of solubility and diffusion velocity proved to be selective, thus for instance both are larger for Na^+ due to its larger hydrate shell than for K^+. Consequently also the bound water contri-

butes to the decrease of the energy of the active transport required to maintain the migration, and to help to preserve the concentration gradient.

In the course of stimulation the changes occurring within the cell and its environment lead to the transformation of the conformation (phase transition) of the constituents, mainly the proteins. As a result, the ion-binding capacity of these constituents as well as the structure of the water undergo an abrupt change. Such effects may be produced for instance by the potential changes of 10^2–10^3 V/s during depolarization. These sudden changes (jumps) explain the phenomena related to the depolarization branch of the action potential. The conformation characteristics of the resting state and the concomitant restitution of the ion-binding capacity satisfactorily explain the depolarization period.

(b) In the phenomenon of the resting and especially the action potential the *ion transport,* mainly the sodium and potassium transport, and the *transport regulation* play an important role. Interesting information can be obtained about the most important molecular mechanisms of these processes from the model membranes, since, according to experience, the permeability of the lipid membranes for individual ions modified by suitable oligo- and polypeptides (as for instance the antibiotic Gramicidin) increases considerably. The increase in permeability is related to the so-called *channel-producing* property of the protein (peptide). According to the model a specific channel operates for every ion. The channels exist in one of two states: permeable or impermeable. In resting cell membranes both the sodium and potassium channels are with high probability in the impermeable state. In case of the change of the electric field due to the stimulatory processes, however (cf. section 6.2.1), the channels open, i.e. attain a permeable state resulting in the increased transport of both ions. According to the model the membrane potential dependence of I_{Na} and I_K described in [6.9] is quantitatively characterized exactly by the probability of the permeable and impermeable state. At the molecular level the permeability changes of the channels are interrelated with the conformation changes of their constituent proteins (peptides). Some 10 to 100 channels are found on a membrane surface of 1 μm^2, which means that the distance between these channels is approximately 0.1 μm. When one single channel opens, an electric current of the magnitude of picoamperes may be measured, which corresponds to the transport of 10^7 ions per second.

6.2.3. Propagation of the action potential

The action potential propagates with a definite velocity and a nearly unchanged amplitude from the site of triggering along the muscle (nerve) fibre. In the propagation of the action potential a part is played by the fact that at a given point of the membrane an action potential is produced whenever the resting potential reaches or exceeds the depolarization threshold level. The action potential maximum appearing at a given point of a muscle fibre is reduced to about one-third of its initial value at a

distance of the membrane length constant. This is quite sufficient to trigger an action potential at this more distant site too.

Table 6.3 shows that from the viewpoint of the propagation mechanism the situation for the squid giant axon, with a diameter of ca. 0.5 mm, is more favourable; if the length constant is sufficiently large, the velocity of propagation may attain even 10–20 m/s. In higher living organisms, the fibres generally have smaller diameters. However, the propagation of excitation may still be faster, due to the well-insulating myelin sheath of the nerve fibres. This is explained by the resistance of the membrane which, due to the presence of the myelin layer (between the Ranvier nodes), is extremely high and consequently the action potential propagates with practically no time loss ($\lambda \sim \sqrt{R_m/R_i}$). This is the so-called saltatory conduction.

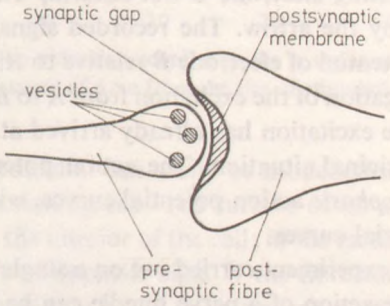

Fig. 6.12. Diagram relating to the synapse

If a nerve fibre is excited in the middle, the excitation propagates in both directions from the point of excitation. However, the normal function of the nervous system is to transport the information carried by the excitation from a given point in the organism to another one. This function in the nervous system is based on the presence of a rectifying system. The rectification is carried out most frequently mediated by means of chemical substances, in morphological and functional units, the synapses, which are specialized to interlink the excitable cells. The synapse between two nerve cells is shown in Fig. 6.12. The synaptic gap, with a width of approximately 20 nm, is situated between the ends of the pre- and postsynaptic fibres. In the presynaptic end are the *synaptic vesicles*. In the transmission of excitation the *neurotransmitter substance* (e.g. acetylcholine, norepinephrine) produced in the presynaptic fibre plays an essential role. The membrane of a given postsynaptic fibre is sensitive only to its respective neurotransmitter molecule. As regards their functions, two types of synapses exist: *excitatory* and *inhibitory* synapses.

The mechanism of synaptic transmission is the following: under the effect of the action potential reaching the presynaptic end the neurotransmitter passes into the synaptic gap. From here its molecules diffuse to the postsynaptic membrane and change the membrane conductivity for Na^+ ions. As a result of the Na^+ ion flux,

the potential of the postsynaptic membrane changes: this is called *postsynaptic potential*. In the case of excitatory synapses this involves depolarization and in inhibitory ones hyperpolarization. According to experience the postsynaptic membrane contains a particularly high amount of proteins. The transmitter substance changes the conformation of these proteins and thus enhances the permeability of the ion channels. As a consequence of the break-down of the transmitter substance the original state of the channels is restored.

6.2.4. Action potential of fibre bundles. Dipole model

Figure 6.13 presents a situation with both measuring electrodes on the *surface* of the fibre. (The stimulating electrode is not shown.) The direction of excitation propagation is denoted by the arrow. The recorded signal is shown in Fig. 6.13*b*. The ordinate gives the potential of electrode *B* relative to *A*. Section I–II of the curve corresponds to the propagation of the excitation from *A* to *B*, and point II is associated with the case when the excitation has already arrived at *B*. Section II–III depicts the restoration of the original situation. The action potential curves presented in Figs 6.4 and 6.7 are *monophasic* action potential curves, whereas those in Fig. 6.13*b* are *biphasic* action potential curves.

Figure 6.13 depicts an experiment carried out on a single fibre, though the voltage signal associated with the action of a nerve bundle can be studied in a similar way. In this latter case the recorded curve usually contains several maxima, as a consequence of the various propagating velocities of the action potentials in the individual fibres of the bundle. Figure 6.14 shows the action potential recorded from cat n. saphenus, which consists of about 2600 fibres. These can be divided into four main groups, depending on their diameters. Since the velocity of propagation varies (among others) with the fibre diameter, instead of one action potential maximum four different maxima (denoted by α, β, γ and δ) can be detected on an electrode placed at a sufficiently large distance from the site of excitation. The curve may be regarded as the *resultant* of the action potentials propagating with different velocities in the individual fibres.

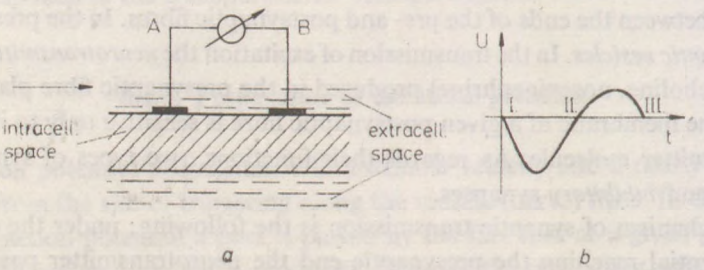

Fig. 6.13. Diagram relating to recording of the biphasic action potential (*a*) and the recorded action potential (*b*)

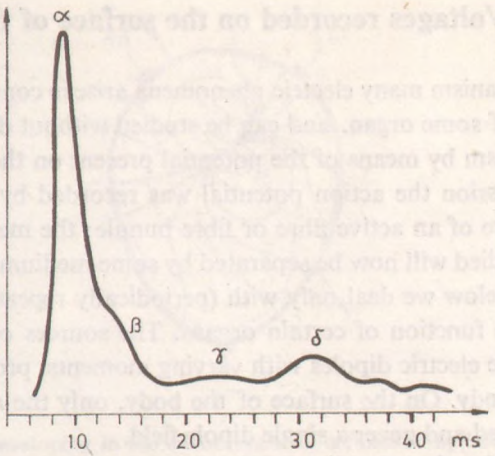

Fig. 6.14. Action potential recorded on the n. saphenus of the cat at a distance of 6 cm from the site of stimulation

The biphasic action potential can easily be modelled with an electric dipole. Let us consider a single functioning cell. The surface of its active part is at a negative potential with respect to the interior of the cell; in the resting part, on the other hand, the potential difference is of opposite sign. If the fibre surface is studied, a varying electric field can be observed on it and in its environment. Figure 6.15 illustrates the moment when the voltage change associated with the excitation process propagating in the direction of the arrow reaches the $A-A'$ plane. An electric field exists between the two sides of the fibre divided by the plane. The lines of force are also depicted. The field is similar to the field of a dipole, the dipole moment pointing in the direction of excitation propagation. In the process of restoration the fibre behaves as a propagating dipole, but the direction of the dipole moment is now reversed.

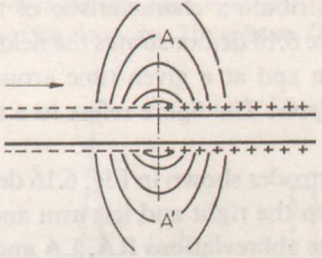

Fig. 6.15. An active nerve fibre as a dipole
In the presented case the excitation propagating in the direction of the arrow has reached the $A-A'$ plane. The diagram also shows the lines of force of the dipole electric field

6.3. Voltages recorded on the surface of the body

In the living organism many electric phenomena arise in connection with the physiological activity of some organ, and can be studied without disturbing the integrity of the living organism by means of the potential present on the surface of the body. In the above discussion the action potential was recorded by electrodes placed directly on the surface of an active fibre or fibre bundle; the measuring electrode and the organ to be studied will now be separated by some medium of a certain thickness (various tissues). Below we deal only with (periodically repeated) potential changes associated with the function of certain organs. The sources of the electric fields in the human body are electric dipoles with varying moments, produced by functioning organs inside the body. On the surface of the body, only the *resultant field of many dipoles* can be studied and never a single dipole field.

In medical practice the recording of the voltages associated with the function of the heart, the central nervous system, the skeletal muscles and with vision are of the greatest interest.

6.3.1. Electrocardiography

The title of this chapter refers to a procedure used in medical diagnosis, which is based on the measurement of electric potentials resulting from the function of the heart on the surface of the body. In this chapter the more important physical aspects of electrocardiography will be dealt with (cf. also section 5.6).

The state of excitation of the heart changes in a rather complex way both in space and time. Close to the heart (e.g. over the epicardium) the spatial distribution of the potential reveals as potential sources the individual parts of the myocardium which are characteristic components of the resulting total potential. Somewhat removed from the heart (at a distance comparable with the dimensions of the heart) the details revealing the components reflecting the individual parts of the myocardium are blurred, and a potential distribution characteristic of the resultant dipole moment becomes predominant. Figure 6.16 demonstrates the field and potential distribution as developed in a given section and at a given time around the heart simulated by a single so-called *equivalent dipole*. The figure refers to a simplified situation.

1. Electric leads. The electrodes shown in Fig. 6.16 denote the usual standard limb electrodes; they are placed on the right and left arm and on the left foot. These are denoted in the diagram by the abbreviations RA, LA and LF, respectively. The limbs are not included in the figure, for they are so far from the heart that the voltage appearing at their proximal end does not decrease appreciably towards the distal parts. Let us connect any two of the three electrodes on the limbs to the voltage-recording apparatus. The *electrocardiogram* is the curve showing the change of voltage in time. In the course of a single heart cycle, maxima (P, R, T) and minima (Q, Z), are recorded

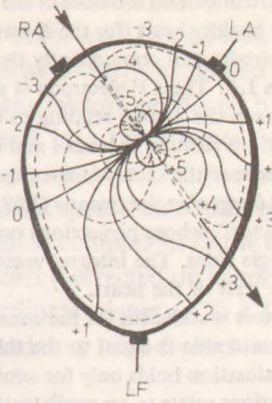

Fig. 6.16. Electric field developing in the environment of a functioning heart as an electric dipole (frontal section of trunk model)

The dotted lines denote the lines of force, and the continuous ones the equipotential surfaces. The direction of the dipole moment is shown by the arrow. The potential of the equipotential surface perpendicular to the dipole moment is arbitrarily taken as zero; the potential values of the other surfaces are denoted by positive or negative numbers (*Katz*, 1937)

as depicted in Fig. 6.17. The former are the positive, and the latter the negative waves. In the first case the heart apex is at a positive potential relative to the heart base, while the situation is reversed in the second case.

Of the three standard limb electrodes, two can be connected in three different ways. Lead I connects the left and right arms, lead II the right arm and the left foot, and lead III the left arm and the left foot. In all three cases curves essentially similar to the first one are obtained, though the three curves are not independent of one another, since the algebraic sum of the voltages is always zero. It follows that if two of the three curves are known, the third can always be constructed.

In the evaluation of electrocardiograms, concepts defined by certain geometric consideration are frequently used. Figure 6.18 demonstrates *Einthoven's triangle*. This is an equilateral triangle whose apices denote the positions of the electrodes. The voltages U_1, U_2 and U_3 at a given time (asso-

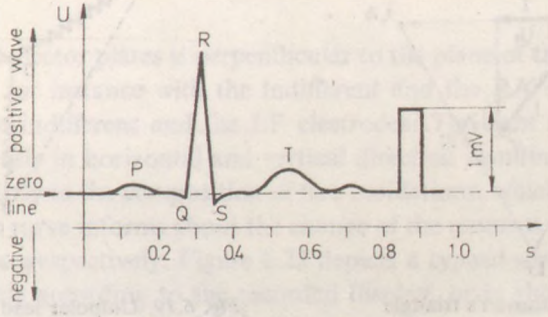

Fig. 6.17. A typical electrocardiogram

ciated with the *R* wave for instance), are drawn on the sides of the triangle. By convention the arrows point to the positive electrode. With a healthy heart (for the *R* wave) the LA electrode is at a positive potential with respect to the RA electrode, and consequently the arrow on the side of the triangle representing lead I will point towards LA. The LF electrode is at a positive potential with respect to both LA and RA, and for this reason the arrows relating to leads II and III point towards LF. The sum of the lengths of the line sections relating to leads I and III is equal to the length of the line section relating to lead II, which expresses the fact that the algebraic sum of the voltages is zero. With the aid of the diagram, the heart *integral vector* (means *QRS* vector), denoted by *I*, can be constructed in a simple way. This is a "vector", whose projections on the triangle sides are equal to the "voltage vectors" drawn on the triangle sides. The integral vector associated with the *R* wave, i.e. the largest vector, is the *main electric axis* of the heart.

The knowledge of two voltage vectors is sufficient for the construction of the integral vector, and the projection of this vector on the third side is equal to the third voltage vector. Without proof, however, it should be noted that this situation holds only for equilateral triangles, and this is clearly the reason why Einthoven's considerations relate to an equilateral triangle.

Besides the standard limb leads, use is frequently made in medical practice of one electrode placed at an active point of the chest, while the other electrode is connected to a point of *constant potential*. The former is the *active*, and the latter the *indifferent* electrode. In the standard limb leads both electrodes are active electrodes and in this case the leads are *bipolar*. On the other hand, if one electrode is kept at a constant potential, the lead will be *unipolar*. Similar electrode arrangements with the same nomenclature are also used, in cases other than electrocardiography.

The indifferent electrode in electrocardiography is usually the *Wilson central terminal*. This is denoted in Fig. 6.19 by the point 0, which is obtained if the RA, LA and LF electrodes are connected through equal resistances (5000—10,000 Ω). In experience and also from the relevant theoretical considerations, the value of the potential at 0 is practically constant. In electrocardiography the so-called *12-lead system* is frequently used. In this system besides the three standard limb electrodes, three further unipolar limb electrodes (aVR, aVL, aVF) as well as six chest leads are applied.

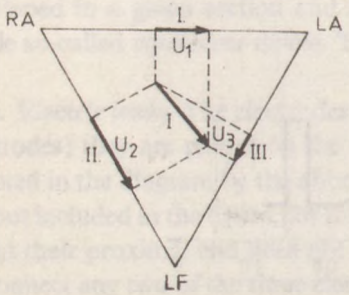

Fig. 6.18. Einthoven's triangle with the integral vector *I*

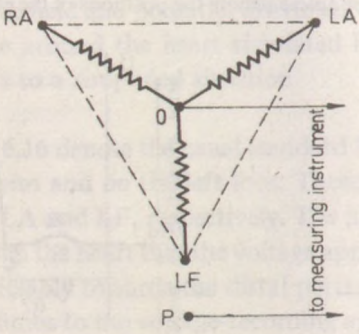

Fig. 6.19. Unipolar lead according to Wilson

2. Special methods. The methods discussed in the following section extend the possibilities of the standard limb, and the 12-lead systems.

(a) *Vectorcardiography* — as already explained by its name — determines the resultant of the dipole moment vectors produced by the heart. This vector changes continuously. Placing the heart in a three-dimensional coordinate system in space (Fig. 6.20) the x, y and z components of the resultant vector are measured. For the purpose of these measurements a special electrode system has been developed, which detects uniformly each individual component of the elementary dipoles independently of their position within the heart (corrected orthogonal leads). The vector is displayed by its projection on the coordinate planes (XY, YZ, XZ). Figure 6.21 depicting the deflector plate pairs of a cathode-ray tube demonstrates the principles of this method.

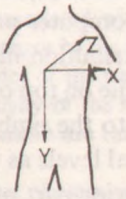

Fig. 6.20. Three-dimensional coordinate system fitted to the body. Diagram relating to stereocardiography

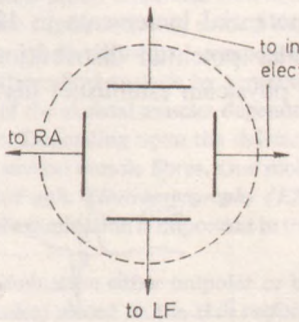

Fig. 6.21. Diagram relating to vectorcardiography

The plane of the deflector plates is perpendicular to the plane of the paper. One plate pair is connected for instance with the indifferent and the RA electrodes, whereas the other with the indifferent and the LF electrodes. The light spot on the screen moves simultaneously in horizontal and vertical direction resulting in a closed plane curve (loop), similarly to the composition of two oscillations, which are perpendicular to each other. The curve informs about the change of the moment as projected on the XY and XZ planes, respectively. Figure 6.22 depicts a typical record. The physician makes his diagnosis according to the recorded display, or in the knowledge of the resultant dipole moment vector calculated by computer.

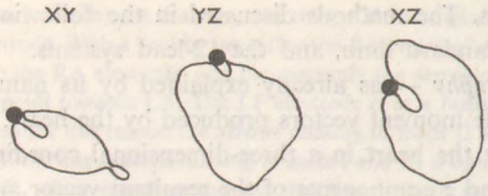

Fig. 6.22. Vectorcardiograms in the frontal (*XY*), sagittal (*YZ*) and horizontal (*XZ*) planes. The larger loop relates to the *QRS* waves and the smaller one to the *T* wave

(b) The knowledge of the *potential distribution on the surface of the body* gives a nearly total information about cardiac action. The potential values are determined by a large number of electrodes (60–250) placed at various parts of the trunk. The results are recorded in the form of computer processed potential distribution maps.

Figure 6.23 shows a potential distribution map, representing the whole body laid out in a single plane. The horizontal line on top of the figure is the clavicle (manubrium sterni), the bottom line corresponds to the umbilical region. On the left side (ab) of the figure are represented the potential levels as measured on the frontal part, and on the right side (cd) those on the posterior part of the trunk. The thin, broken line on the ECG curve in the upper right corner of the figure indicates the time when the potential map has been determined. The contour lines connect the points of identical potential. The dotted line represents the zero potential level, while the other contour lines indicate the symmetrical potential increments in 120 μV steps, as related to the Wilson reference lead. Similar potential distribution maps record the other phases of cardiac function. The physician establishes his diagnosis on the basis of the potential maps.

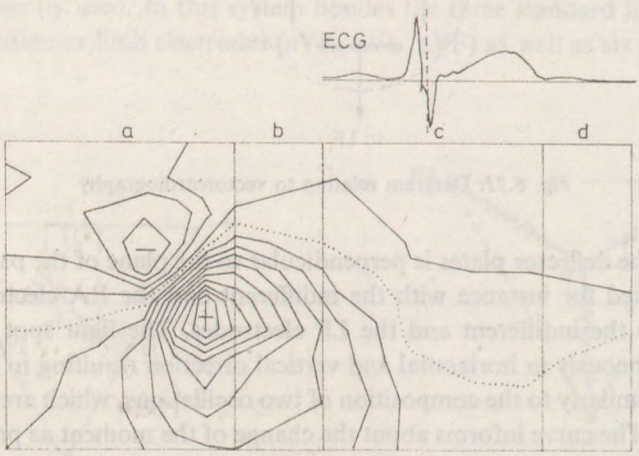

Fig. 6.23. A potential distribution map on the body surface. The time of determination is indicated by the thin dashed line in the ECG curve. Signs + and − denote the sites of highest positive and negative potentials, respectively

6.3.2. Potentials connected with cerebral and muscular functions and with light sensation

1. Potential changes produced by the brain or its various regions can also be recorded. These changes are the *macrorhythm*, which differs considerably from the *microrhythm* produced by the action potentials appearing in the individual functioning nerve cells. Actually, the macrorhythm is the resultant of the action potentials of the cerebral neurons. The method of examining the macrorhythm associated with the function of the central nervous system is *electroencephalography (EEG)*, and the recorded curves are *electroencephalograms.*

For this type of examination one active and one indifferent electrode are used. The active electrode is placed either on the appropriate point of the skull or (in a surgical operation) directly on the cerebral cortex (this latter method is *electrocorticography*). For the indifferent electrode some inactive site, e.g. the ear-lobe or the Wilson central terminal, is selected.

The recorded electric signals (waves) associated with the cerebral functions are characterized either by the *frequency* of the potential change, or by the value (amplitude) of the potential difference. Under physiological conditions, the frequency of the waves lies in the range 2–40 Hz, with amplitudes of 20–80 μV, depending upon the activity of the nervous system (wakefulness, sleep, etc.). In pathological cases the characteristic frequencies are rather low, whereas the wave amplitude may amount to several hundred μV.

Experience shows that, in the evaluation of electroencephalograms, information about the cerebral functions can be obtained, mainly from the differences between the macrorhythms of the various areas. For a correct assessment of the differences, simultaneous electroencephalograms are generally recorded for several cerebral areas. (In clinical practice 12–16-channel EEG equipment is used.) This type of evaluation is purely empirical. In a normal state, the records in the individual channels generally consist of the superposition of several waves of different frequencies and amplitudes (Fig. 6.24). The separation of the individual components with definite frequencies and amplitudes requires appropriate mathematical analysis by computer.

2. The voluntary function of the skeletal muscles depends upon the functions of the motor nerve and the nerve–muscle junction. Depending upon the delicacy of the function of a given muscle, a single motor nerve innervates several muscle fibres. One motor neuron together with its innervated muscle fibre constitutes a motor unit. *Electromyography (EMG)* studies functions connected with the motor units. This method of examination is important in the functional diagnosis of the peripheral nerves.

For the purpose of this examination either unipolar or bipolar leads are used. In the case of a measuring electrode (or electrodes) placed on the *skin surface*, the resultant of the action potentials of several motor units is recorded; with a needle electrode, on the other hand, the propagation of the

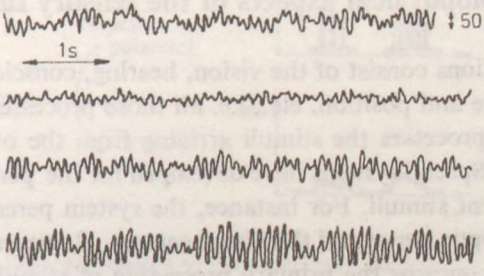

Fig. 6.24. Electroencephalograms

excitation in one nerve fibre and its transmission from the motor neuron to a muscle fibre may be examined separately in a single motor unit. The excitation processes of the motor nerve are frequently triggered by a square-wave generator connected to the recorder (cf. section 5.3.3), which also allows the determination of the propagation velocity of the excitation.

3. The action potentials induced by illuminating the retina are examined by *electroretinography (ERG)*. The recording electrode is usually placed directly on the frontal surface of the eye, on the cornea. In this way voltage amplitudes of 20–300 μV are obtained. The electroretinogram is produced as the resultant of several voltage components varying in time; the maxima obtained are usually called the *a*, *b*, *c*, *d* waves. The shape of the electroretinogram (mainly of the *a* and *b* waves), i.e. the amplitude of the waves, their duration and their appearance after the start of the illumination are found to depend strongly upon the conditions of the examination (the degree of dark adaptation, the duration and intensity of illumination, etc.) which raises the necessity of standardization.

In this section, only certain, special problems associated with the recording of the action potentials (e.g. the construction of the measuring electrodes) have been dealt with, since the technical problems of signal shape analysis were discussed in more detail in section 5.6.1. Here we give only some informative data which may help in the selection of the electric equipment to be used to record the action potentials. Table 6.4 summarizes some of the more important data characteristic of the action potentials discussed. For the sake of comparison, the Table contains the same data on the action potential of a single cell. The frequency data were obtained by Fourier analysis of the respective curves.

Table 6.4

Characteristic bioelectric potential data

Action potential	Frequency range (Hz)	Voltage (mV)	Notes
A single cell	0 –10,000	50 –130	Monophasic action potential
Electrocardiography	0.1–200	0.1–3	
Electroencephalography	1 –70	0.001–0.1	
Electrocorticography	10 –100	0.01–0.1	
Electromyography	10 –1000	0.1–5	Surface electrode
Electromyography	10 –10,000	0.05–5	Needle electrode
Electroretinography	0.1–100	0.02–0.3	

6.4. Biophysical aspects of the sensory functions

The sensory functions consist of the vision, hearing, conscious and subconscious sensations of pressure and position, etc., i.e. all those processes in general by which the living organism processes the stimuli arriving from the outside world or from inside the organism. Special systems have developed for the perception and the processing of the different stimuli. For instance, the system perceiving and processing light is the eye, the optic nerve and the visual centres. However, the various systems are specific only as concerns the primary processing of stimulus energies, i.e. their transformation. Subsequent to this process, every type of stimulus uniformly produces

action potentials in the respective sensory nerves. The type of the energy (mechanical, electromagnetic, chemical, etc.) appearing as a stimulus, the intensity of the stimulus and its change and spatial distribution are expressed only in the localization of the action potential and in the parameters characterizing its changes.

6.4.1. Sensory functions in general

The sensory functions are discussed in this section without emphasizing the specific details; only the general aspects are surveyed. As regards the principles of operation, every system associated with the sensory functions may be modelled as an *analogue signal processing system* (cf. section 5.6.1). In this survey, only the aspects of signal processing are considered and the discussion is restricted to processes occurring in the periphery. With the aid of Fig. 6.25 let us follow the chain of processes induced in the receptor cell by the stimulus, and the quantitative relations between the individual elements of the chain.

1. Signal transduction. Physical or chemical stimuli manifest their effects primarily in the *receptor cells* (or in a part of them). The receptor cell is either directly exposed to the stimulus, or is localized in a structure which makes full use of the stimulus energy and transmits it without loss. In the latter case the stimulus affects the cells indirectly. The direct case is observed, for instance, in connection with the stretch receptors of the muscles, whereas the indirect case is typical for the sense organs. The *transduction of stimulus signals into electric signals* is a general function of receptor cells, or more exactly, of the specialized membrane parts of these cells. Thus, light

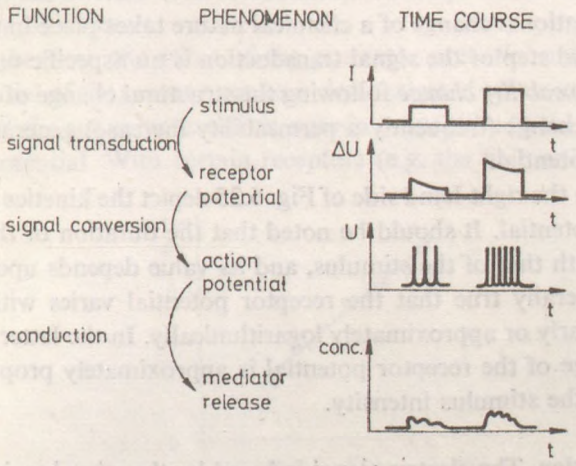

Fig. 6.25. Diagram relating to the function of the receptor cell
Time course of processes; *a:* variation of the stimulus intensity, *b:* variation of the receptor potential; *c:* action potential pulses; *d:* variation of the synaptic mediator substance concentration

is transduced in the rods and cones of the retina, and sound in the hair cells of the cochlea. Accordingly, the receptor cells act as transducers. In response to the effect of the stimulus, the resting potential of the receptor cells changes considerably; this change is called the *receptor potential.* The transducer function of the receptor cell, and with it the relation between the receptor potential and the resting potential, will be demonstrated in a simple example for the case of the *stretch receptors of the muscle.* For these receptors an adequate stimulus is the stretching of the muscle fibres, which, as already mentioned, has a direct effect. As a consequence of mechanical stretching, a local depolarization is induced on the membrane of the receptor cell; with regard to the electric model of the membrane (cf. Fig. 6.5) this may be interpreted in the following way. The stretching increases the membrane surface area, and decreases its thickness (the volume of the membrane remains constant). Both dimensional changes result in an increase of the capacitance. The same dimensional changes simultaneously increase the membrane permeability, which in turn decreases the membrane cross-resistance (as concerns only the dimensional changes, the increase of the permeability is aspecific, since it holds for each ion). According to the model the capacitance increase induces a charge flow. The charges are supplied by the e.m.f. of the membrane potential, and the induced current flows through a decreased resistance. It is quite clear that during this process the potential difference between the two plates of the capacitor is smaller than at rest, which corresponds to the depolarization observable in the given case. The resting potential is restored only when the capacitor is recharged.

It may be observed in this example, and it is also generally true, that the transducer function consists of two steps. In the first step the stimulus affects only the molecules specific for it, and in the course of this step the *energy of the stimulus* undergoes primary *transformation.* In the stretch receptor, for instance, this means simply a mechanical change in the receptor membrane structure, while in the case of the light receptor a conformational change of a chemical nature takes place in the photolabile pigment. The second step of the signal transduction is an aspecific one and in every case involves a *permeability change* following the structural change of the membrane. This permeability change (frequently a permeability increase) generates ionic fluxes and the receptor potential.

Lines *a* and *b* on the right-hand side of Fig. 6.25 depict the kinetics of the stimulus and the induced potential. It should be noted that the duration of the receptor potential coincides with that of the stimulus, and its value depends upon the stimulus intensity. It is generally true that the receptor potential varies with the stimulus intensity either linearly or approximately logarithmically. In the latter case it may be said that the change of the receptor potential is approximately proportional to the relative change of the stimulus intensity.

2. Signal conversion. The electric signal induced by the stimulus, i.e. the receptor potential, is converted as the next step in the processing. This transformation is an *analogue–analogue conversion* (cf. section 5.6.1). The new signal is also an electric

one; it is the *action potential* of the sensory nerve associated with the receptor cell. The action potential is initiated by the receptor potential in such a way that it affects the corresponding membrane part of the sensory neuron as a stimulus. Consequently, in the sensory function the receptor potential plays the same role as, for instance, the square-wave current pulse in the artificial excitation of a nerve. Because of this function the receptor potential is also called the *generator potential.*

The generator potential may either increase or decrease the resting potential of the sensory nerve, and may generate hyper- or depolarization, respectively. The electric properties of the membrane are locally changed by a hyperpolarization and by a depolarization which does not attain the threshold level. For the generation of the action potential and for signal transmission it is necessary that the value of the generator potential should be at least as high as the threshold level of the respective membrane part. The stimulus must be strong enough to induce a sufficiently large generator potential. The smallest stimulus intensity whose generator potential can induce an action potential on the sensory nerve is the *threshold intensity.*

3. Conduction. Consider Fig. 6.25 once more. The diagram depicts a long-lasting generator potential which generates several action potentials in succession in the sensory fibre. It is seen that the frequency of the action potential series is the higher, the larger the generator potential, i.e. the stronger the stimulus. All this can be understood on the basis of the electric properties of the excitable cell, since in the course of the action potential the depolarization threshold level decreases after the potential peak from a relatively large value to the value associated with the resting state (cf. section 6.2.1); consequently, the larger the generator potential, the sooner the next action potential can be started. The possible upper limit of the action potential frequency, as determined by the duration of the absolute inexcitability of the nerve, is approximately 1000 Hz.

The relation between the value of the generator potential and the frequency of the action potential of the sensory nerves is demonstrated by an example in Fig. 6.26. The frequency of the action potential changes in proportion to the change in value of the generator potential. With certain receptors (e.g. the hair cells in the labyrinth)

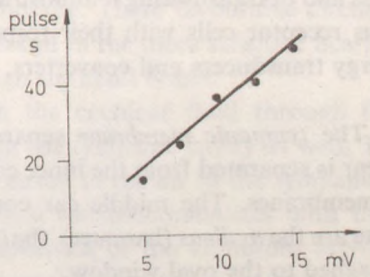

Fig. 6.26. Relation between the magnitude of the generator potential and the frequency of the action potentials generated in the stretch receptor of crab muscle

the action potentials are generated with a constant frequency on the nerve, even without the effect of a generator potential. This frequency is increased or decreased by the depolarization or the hyperpolarization caused by the generator potential. However, it is true in these cases too that the higher the change of the generator potential, the higher the change of the frequency of the action potential. If the relations between the generator potential and the stimulus intensity and between the generator potential and the frequency of the action potential are compared, it is found, in agreement with experience, that the frequency change of the action potential is nearly proportional to the relative intensity change of the stimulus. This finding, which is generally valid for the sensory functions, is the basis of the Weber–Fechner law (cf. section 5.4.1).

Let us return to Fig. 6.25. The last chain-link of the receptor cell function is the synaptic transmission of the stimulus by the liberation of the relevant synaptic mediator substance. The further processing (to produce sensation) occurs at the appropriate sites in the central nervous system, where the action potential series propagating along the sensory nerve fibres finally arrive.

It should also be mentioned that in signal processing the *amplification* of the signal is an important intermediate process. Amplification connected with the sensory function is carried out in the course of the signal transduction and conversion. The energy necessary for the signal amplification is provided by the metabolic processes of the cells.

6.4.2. Hearing (as an example of sensory function)

In the following, a relatively well-understood sensory function, hearing, is presented in detail as an example. The discussion will include only those physical phenomena which are associated with the development of the sound sensation by the processes occurring in the ear. The organ of hearing is the ear (Fig. 6.27), which perceives mechanical vibrations (sounds) in a well-defined frequency range (20–20,000 Hz). The special structural units of the ear fulfil the functions of receiving the energy of the sound stimulus and of transmitting it almost without loss to the receptor cells. The ear also contains receptor cells with their respective nerve fibres. These latter structures act as energy transducers and converters.

1. Energy transmission. The *tympanic membrane* separates the *middle ear* from the outer ear. The middle ear is separated from the inner ear by an oval and a round window covered by thin membranes. The middle ear contains three small bones, the middle ear ossicles: these are the *malleus* (hammer), the *incus* (anvil) and the *stapes* (stirrup), whose base is attached to the oval window.

The sound vibrations arriving from the air are transmitted by the middle ear with only minimal energy loss to the inner ear, or more exactly to the fluid (endo-

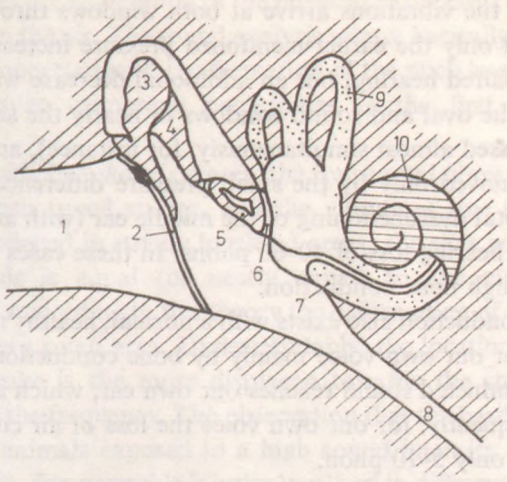

Fig. 6.27. The structure of the ear
1: outer auditory canal; *2:* tympanic membrane (eardrum); *3:* hammer; *4:* anvil; *5:* stapes; *6:* oval window; *7:* round window; *8:* Eustachian tube; *9:* semicircular canals; *10:* cochlea

and perilymph) in the coiled tube-like organ, the *cochlea*. For a given sound wave the vibrational amplitude is smaller in a liquid than in the air. This holds for the ear too. Under these circumstances the energy transmission will be favourable if a higher pressure is transmitted to the inner ear than the pressure actually present in the open air or on the tympanum. In fact, an amplification occurs in the middle ear in which both the tympanic membrane and the ossicles participate. The pressure increase results partly from the fact that, while the surface area of the tympanic membrane part to which the handle of the hammer is rigidly attached is ca. 55 mm², the stapes footplate has a surface area of only 3.2 mm². The same force is distributed first on the greater, and subsequently on the smaller surface area, which corresponds to an approximately 17-fold pressure amplification. Another reason for the pressure increase is the lever system formed by the ossicles; the ratio of the lever arms is 1.3:1. As a result of the middle ear function, the pressure on the stapes footplate is 20–22 times that on the tympanic membrane. These favourable circumstances of energy transmission in the middle ear result in the more sensitive hearing of *air-conducted* sound than that conducted through the skull bones.

Pressure changes reach the cochlear fluid through the tympanic membrane–ossicles system and through the tympanic cavity as well. The vibrations of the tympanic membrane are transmitted to the air in the tympanic cavity, and through this to the round window, which intercommunicates with the fluid of the inner ear. However, the pressure amplitudes of the vibrations arriving at the round window are approximately 20 times smaller than those on the oval window, and consequently their role can be neglected in the case of a healthy middle ear. Nevertheless, the situation is quite different if the tympanic membrane and the auditory ossicles are

missing. In this case the vibrations arrive at both windows through the air. Under these conditions, not only the earlier-mentioned pressure increase is absent, which clearly results in impaired hearing, but an additional decrease will occur due to the vibrations reaching the oval and round windows in nearly the same phase (the two windows are compressed almost simultaneously, for instance), and consequently the endolymph will be moved only by the small pressure difference arriving from the two windows. The total dysfunctioning of the middle ear (with an otherwise healthy inner ear) leads to a hearing loss of 40–60 phons. In these cases hearing is achieved practically only through bone conduction.

Naturally, bone conduction also exists with a normal, healthy middle ear function. For example, we hear our own voice mainly by bone conduction; only a small proportion of the air-conducted sound reaches our own ear, which is shielded from the voice sounds. Consequently, for our own voice the loss of air conduction results in a sound decrease of only 5–10 phon.

2. Analysis of the mechanical stimulus in the cochlea. The transducer and converter functions take place in the cochlea, which communicates with the middle ear through the oval and the round window. The cochlea is a coiled tube with two and a half turns and with a narrowing membranous channel at its end. The cochlea is divided into three parts by a partly bony, partly fibrous wall (Fig. 6.28). The elastic fibrous separating wall is the *basilar membrane,* and the thinner wall is *Reissner's membrane.* The former is mainly important for hearing. Its width increases from 0.04 mm to 0.5 mm from the oval window towards the apex of the cochlea. On the basilar membrane is the *organ of Corti,* which contains the endings of the auditory nerve fibres. These are connected with elongated cells covered on top with hair, the hair cells, which are further covered with the *tectorial membrane.* The vibrations arriving from the middle ear to the cochlear fluid are transmitted from here to the tectorial membrane and Reissner's membrane.

In connection with the functions of the sense organs, including those of hearing, it is essential to understand the analysis of the physical effect. In the present case

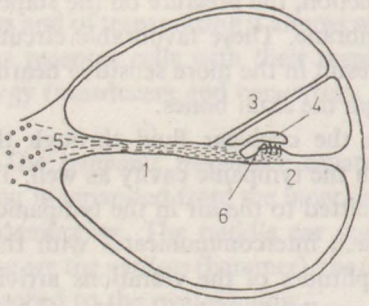

Fig. 6.28. Schematic diagram of the cochlea
1: lamina spiralis ossea; *2:* basilar membrane with the organ of Corti; *3:* Reissner's membrane; *4:* tectorial membrane; *5:* fibres of auditory nerve; *6:* hair cells

this analysis involves the recognition of the physical parameters of the sound stimulus and its processing in the air. The sound analysis relates basically to the frequency and intensity, i.e. it is connected with the formation of the pitch level and sound intensity sensation. The basilar membrane participates in the first step of the analysis (Table 6.5).

In accordance with *Helmholtz's theory*, the transversal fibres of the basilar membrane act as variously tuned strings, and the vibrations due to sounds of various frequencies are produced in strictly localized areas: the sites where the frequency of the exciting sounds is equal (or nearly equal) to the eigenfrequency of the string *(resonance)*. According to this theory the deformation of the basilar membrane is restricted to a very small area. Understandably, the location of the resonance on the basilar membrane is the more displaced towards the stapes (i.e. the shorter chords), the higher the frequency. The observation that the basilar membrane and the organ of Corti of animals exposed to a high sound intensity for a prolonged time exhibit histologically demonstrable injuries localized in different frequency-dependent areas is in agreement with the Helmholtz theory. However, it is difficult to conceive the sharp resonance required by the theory if the histological structure (interstitially embedded tissue fibres) of the basilar membrane is considered, together with the circumstance that its vibrations are strongly damped by the highly viscous endolymph surrounding the basilar membrane.

In model and cadaver experiments, *Békésy* investigated the vibrations of the basilar membrane and hence gave a satisfactory explanation of the frequency and intensity analysis. He found that the movement of the stapes, with the mediation of the cochlear fluid, induces travelling *waves* in the basilar membrane, with a frequency equal to the sound frequency. The shape of the travelling waves is influenced not only by the frequency, but also by the elasticity of the membrane, the connections between the fibres, the friction between the basilar membrane and the surrounding medium, etc. The overall result is that the amplitude of the travelling wave varies along the membrane, even at a fixed intensity. Figure 6.29 depicts the vibrations at a given time, and also the amplitude distributions. At low frequencies the maximum amplitude is formed close to the apex of the cochlea, while at sufficiently high frequencies it lies near the oval window. Thus, Békésy's experiments indicate that the frequency-dependence of the location of the maximum amplitude forms the basis of the frequency-analysis, though the maximum is not sharp. The analysis of the sound intensity also takes place in the cochlea, since the amplitude of the mechanical vibrations and the area of the vibrating surface of the basilar membrane depend upon the sound intensity. It is of determining importance in the hearing process that the structures (including the organ of Corti) on the basilar membrane are deformed to various degrees by the vibration of this membrane.

3. Transducer and converter function. The receptor cells are the hair cells, which thus perform the transducer function. The *shear forces* acting on these cells induce

Table 6.5

Appearance and representation of the frequencies and intensities of sound stimuli reaching the ear in the course of peripheral signal processing

Transducer function ↓ | *Converter* function ↓

Middle ear	Basilar membrane	Hair cells	Auditory nerve
	The *frequency* of the strongly damped wave agrees with that of the stimulus	The *frequency* of the *microphone potential* agrees with that of the stimulus	
The *frequency of forced vibration* agrees with that of the stimulus	The position of the *amplitude maximum* depends upon the frequency	The *position* of the *maximum* amplitude of the microphone potential depends upon the frequency	The *frequency distribution* and *the position of the maximum frequency* are characteristic of the stimulus frequency
The *product of the intensity and the area* remains constant	The magnitude of the *amplitude* increases with the intensity of the stimulus The size of *the vibrating region* increases with the intensity	The *amplitude* of the microphone potential increases with the intensity of the stimulus The *region* in which the microphone potential appears increases with the intensity of the stimulus	The *frequency* of the action potential increases with the intensity of the stimulus The *number of active fibres* increases with the intensity of the stimulus

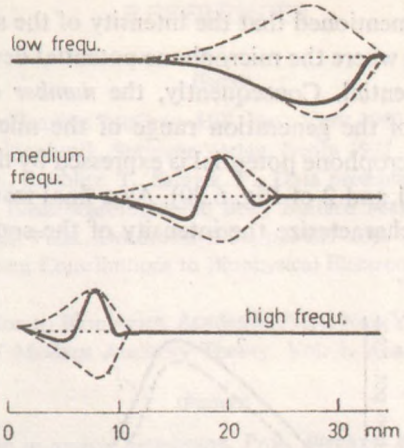

Fig. 6.29. Travelling waves developing on the basilar membrane at various frequencies
The numbers on the horizontal axis denote the distances to the oval window; the dashed lines show the amplitude distribution (envelopes)

the receptor potential characteristic of the hearing process. This is the *microphone potential (cochlear potential)*.

All general statements on the characterization of the receptor potential are also valid for the microphone potential. It is found in practice that the frequency of the microphone potential is equal to the frequency of the sound stimulus. A microphone potential is produced in every hair cell located on the vibrating part of the basilar membrane. The *amplitude* distribution of the potential changes follows the amplitude distribution of the travelling waves produced on the basilar membrane. This means that the maximum of the cochlear potential is found at the maximum vibrating amplitude. Thus, in the case of the microphone potential, similarly as for the basilar membrane, the excitation frequency is expressed partly by the frequency of the potential and partly by the position of the *maximum amplitude*. The *intensity* of the sound stimulus is manifested partly via the *amplitude* of the produced microphone potentials and partly via the *size of the area* where the microphone potential is actually produced.

The microphone potentials generate the action potentials in the auditory nerve endings at the hair cells. The properties of these action potentials are related to the sound stimulus analysis in the following way. For a sound stimulus of given frequency the microphone potential has a characteristic amplitude distribution; accordingly, in the nerve fibres in the environment of the hair cells, the microphone potentials of different amplitudes generate action potential series whose frequency changes from fibre to fibre. The *frequency distribution* of the action potential series and the *localization of the maximum frequency* characterize the frequency of the sound stimulus (curves 1 and 3 of Fig. 6.30).

It has already been mentioned that the intensity of the sound stimulus influences both the size of the area where the microphone potential develops and the magnitude of the microphone potential. Consequently, the *number* of active auditory nerve fibres is characteristic of the generation range of the microphone potential, while the magnitude of the microphone potential is expressed by the frequency of the action potential series (curves 1 and 2 of Fig. 6.30). As a final result, these two parameters of the action potential characterize the intensity of the sound stimulus at the auditory nerve level.

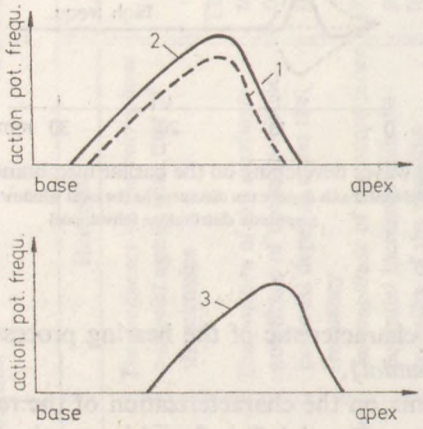

Fig. 6.30. Representation of the intensity and frequency of the sound stimulus reaching the ear in the frequency distribution of the action potentials propagating via the auditory nerve
The horizontal axis represents the positional coordinates of the fibres supplying the basilar membrane (from the base of the cochlea to its apex). *1* and *2:* sounds of the same frequency but different intensities; *2* and *3*: sounds of the same intensity but different frequencies

In order to measure the potentials associated with hearing, one electrode is usually placed on the auditory nerve, and the other on some indifferent area, e.g. the petrous bone. If the active electrode is sufficiently close to the cochlea, the resultant of the two potentials (microphone and action potential) is measured. However, if the electrode is at some distance, the action potential can be recorded separately. The microphone potential can then be determined by comparing the two experimental results (from the difference curves).

The individual steps of the peripheral stimulus analysis occurring in the ear are summarized in Table 6.5.

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7. COMMUNICATION AND CONTROL. THE ELEMENTS OF BIOCYBERNETICS

Cybernetics, one of the most rapidly developing branches of science, at the same time affects the development of practically all other sciences. It has particularly close connections to biology and medicine, and these ties may be expected to become even stronger in the future. *Cybernetics* deals with the problems of *communication (information transmission)* and *control* in highly organized systems (electronic computers, living organisms, factories, etc.), and studies the common structural and functional principles to be found in the different systems. This makes cybernetics an interdisciplinary science; it forms a connecting link between such fields as mathematics, technical sciences, biology, psychology, linguistics, ecology, etc.

Biocybernetics may be thought of as a scientific border territory in which a deeper understanding of biological phenomena and processes is sought with the aid of cybernetics, and in which the discovery of new relations in biological systems is attempted.

7.1. Information transmission

Technical communication or information-transmitting systems include the telegraph, radio and television, while biological examples are the processes of seeing, hearing, etc. In the following sections the common features of communication systems with various structures (information-transmitting chains) will be discussed. Attention will be given to the concept of information as a measurable quantity.

7.1.1. Information-transmitting systems

General structure. The schematic diagram of an information-transmitting system is presented in Fig. 7.1. Information arrives from the information source to the transmitter unit, which not only transforms the information into *signals* suitable for further transmission, but also transmits the signals which carry the information. The assignment of an unequivocal signal series to the information is *coding*. The *code* is the rule relating to the assignment, which allows the reconstruction of the information from the signals. The signals are transmitted to the receiver through a *channel*. Every

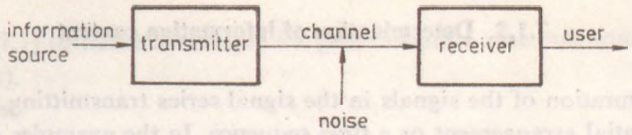

Fig. 7.1. Block diagram of a communication system

system which transmits information serves as a channel. Besides the signals carrying information, signals from the environment may also reach the channel. These latter signals comprise *noise*, which may distort the original meaning of the information, thereby disturbing its correct interpretation. Information processing in which signals are transformed into directly understandable information is carried out in the *receiver*. This latter process is *decoding*.

Some examples of communication systems. As a technical example the radio may be mentioned, where the structures performing the various functions can be well illustrated, and the processes occurring in the system can easily be followed. In a radio broadcasting system the information source is the voice of the speaker, and the transmitter unit corresponds to the radio broadcasting transmitter. The coding includes all those processes which transform the sound vibrations into modulated radiowaves. The radiowaves form the channel and the radio receivers play the role of the decoding unit. This latter device transforms the modulated electromagnetic waves into sound vibrations. The user of the information is the listener.

As a biological example, let us consider hearing (cf. section 6.4.2). In this case the information source is the sound arriving at the ears. The hair cells of the organ of Corti correspond to the transmitter, for in these cells the mechanical vibration is transformed into a microphone potential inducing the action potential of the fibres of the auditory nerve. The coding consists of these processes. Subsequently, the information is carried by action potential signals, the channel being the fibres of the auditory nerve, and the receiver the hearing cortex. The information on the frequency and intensity of the sound stimulus is coded in two ways, involving the position of the active auditory nerve fibres, and also the frequency of the action potential signal of a single fibre.

A further example is from the field of biological processes at a molecular level. In the case of genetic information the information source is the DNA, whose base sequence (cf. section 1.5.4) indicates the information required by the protein-synthetizing apparatus concerning the structure of the protein. The transmitter is the DNA section (gene) corresponding to the protein in question, and the information is coded in the process of transcription. The signal combination suitable for transmission in the given case is the base sequence of the messenger RNA, which arrives through the cytoplasm (the channel) to the receiver, the ribosome. The decoding, i.e. the translation, is carried out here, resulting in a protein with primary (and higher order) structure corresponding to the information induced by the gene.

7.1.2. Determination of information content

The configuration of the signals in the signal series transmitting the information is either a spatial arrangement or a time sequence. In the examples discussed above the base sequence of the DNA molecule represents the first case, and the modulation of the carrier electromagnetic waves in a radio the second possibility. In the process of hearing associated with action potential signals, both arrangements are present.

The quantity of information can be determined exactly in two steps; these are discussed in points 1 and 2 below. Here only one basic idea will be emphasized: a configuration contains the more information, the more unexpected it is. In a different formulation: the information content is the greater, the smaller the probability of occurrence of a given configuration.

1. The uncertainty of the experimental results. The entropy of the experiments. In any given case there are generally various possibilities for the content of a communication, the result of some observation or the outcome of an experiment; the actual content, result or outcome (in general the outcome) is usually not known in advance: the outcome is uncertain. For instance, one cannot tell in advance the number of the lottery ticket which will be drawn, and similarly it is impossible to know in advance in which way the actual DNA molecule will be built up from the four different nucleotide bases. The uncertainty concerning the outcome of the individual experiments and observations (subsequently: experiments) may be characterized quantitatively in the following way.

(a) First we are dealing with an experiment which has k outcomes of *equal probability*. An example of this is lottery ticket drawing, since each ticket participates in the game with the same probability. Clearly, the uncertainty is the greater, the higher the number of possibilities of the outcome. If k equal outcomes of an experiment α are possible, the uncertainty of the experimental result, denoted by $H(\alpha)$, can be characterized by $\log k$, i.e.

$$H(\alpha) = \log k \qquad [7.1]$$

Definition [7.1] expresses the empirical finding that the uncertainty increases with the number of possible outcomes, and the fact too that for one possible outcome the result is beyond doubt, i.e. there is no uncertainty at all: according to [7.1] $H(\alpha)=0$ when $k=1$.

Let us calculate the uncertainty of the nucleotide base sequence for a DNA molecule consisting of 10^6 bases. At any site of the molecule any one of the four different bases may occur, and consequently the number of possible sequences will be 4^{10^6} (repeated variation). Assuming that the probabilities of every possible sequence are equal, [7.1] may be used. In this case $k=4^{10^6}$, so that $H(\alpha)=10^6 \log 4$.

(b) We now discuss experiments whose possible outcomes occur with *different probabilities*. We start from the above special case (equal probabilities of outcomes)

and transform [7.1] to make it suitable for generalization (the outcomes have different probabilities).

It is clear that

$$\log k = \frac{1}{k} \log k + \frac{1}{k} \log k + \ldots + \frac{1}{k} \log k \qquad [7.2a]$$

where the right-hand side consists of the sum of k terms. Since

$$\log k = \log \left(\frac{1}{k} \right)^{-1} = -\log \frac{1}{k}$$

the right-hand side of [7.2a] can also be written in the form

$$\log k = -\frac{1}{k} \log \frac{1}{k} - \frac{1}{k} \log \frac{1}{k} - \ldots - \frac{1}{k} \log \frac{1}{k} \qquad [7.2b]$$

The right-hand side of [7.2b] may be conceived in the following way. Let us denote the possible outcomes of an experiment by $A_1, A_2, \ldots, A_k$, or in brief by A_i, where $i = 1, 2, \ldots, k$, and the probabilities of their occurrence by $P(A_1), P(A_2), \ldots, P(A_k)$, or in brief by $P(A_i)$, where $i = 1, 2, \ldots, k$. If the outcomes A_i occur with equal probabilities, $P(A_i) = \frac{1}{k}$ $(i = 1, 2, \ldots, k)$, and [7.2b] can be written as

$$\log k = H(\alpha) = -P(A_1) \log P(A_1) - P(A_2) \log P(A_2) - \ldots - \ldots - P(A_k) \log P(A_k)$$

or more compactly

$$H(\alpha) = -\sum_{i=1}^{k} P(A_i) \log P(A_i) \qquad [7.3]$$

The right-hand side of [7.3] can also be calculated when the probabilities of the various outcomes are different. The value obtained is termed in every case (on certain physical analogies) the entropy of the experiment. By definition the *uncertainty of the outcome of an experiment* is characterized by the *entropy of the experiment*. Obviously [7.3] includes [7.1] as a special case.

It was assumed in the above DNA example that the possible base sequences occur with equal probabilities. In reality the probabilities of the possible sequences are not equal, and consequently the data obtained via [7.1] do not give the entropy correctly. Our knowledge on the probabilities of the individual configurations is at present still far from complete, but it appears definite that the actual uncertainty is smaller than that obtained from [7.1], since the following statement is true in general: *the most uncertain of all experiments with k outcomes will be that one whose possible outcomes have equal probabilities.*

The correctness of this statement will be demonstrated in the simplest case, when only two outcomes (A_1 and A_2) are possible. Let us denote the probabilities of the outcomes by $P(A_1)$ and $P(A_2)$. Since one of the two outcomes is sure to occur, $P(A_1) + P(A_2) = 1$. Thus, for any value of $P(A_1)$, $P(A_2)$ can be calculated, and from these two data the entropy $H(\alpha)$ of the experiment in question can

be obtained from [7.3]. The results of the calculations are depicted in Fig. 7.2. The uncertainty is indeed maximum at $P(A_1)=P(A_2)=0.5$, when the value of the uncertainty is 0.3 (cf. left-hand ordinate).

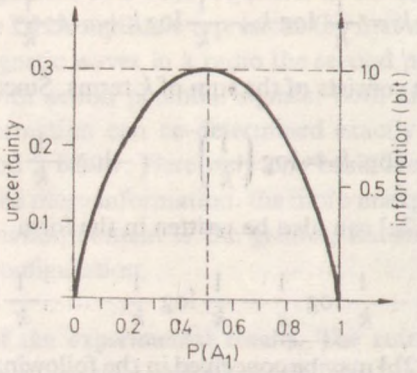

Fig. 7.2. The uncertainty of an experiment with two possible outcomes as a function of the probability of occurrence of one outcome $P(A_1)$
The right-hand ordinate shows the quantity of information to be obtained from experiments with two outcomes

2. Quantity of information. If the expected result is obtained in an experiment, the resulting information is considered to be less than in the event of an unexpected outcome. Consequently, the information is characterized by a quantity which for some outcome A_i of the experiment is the smaller, the larger the probability $P(A_i)$ of the occurrence of A_i, and vice versa. By definition: *the information obtained on the occurrence of an outcome A_i of an experiment is characterized by the quantity* $\log \frac{1}{P(A_i)}$, i.e. by $-\log P(A_i)$.

In practice, not only the individual information quantities of the *individual outcomes* of an experiment are important; the calculations frequently involve the information content of the *respective experiment*. The information content of an experiment is characterized by the average (expected) value of the individual information quantities, which will be shown to be equal to the entropy of the experiment.

Let us denote, as previously, the possible outcomes of an experiment by $A_1, A_2, \ldots, \ldots, A_k$, and the associated probabilities by $P(A_1), P(A_2), \ldots, P(A_k)$. Let us assume that if the experiment is repeated N times, outcome A_1 will occur n_1 times, outcome A_2 n_2 times, ..., and outcome A_k n_k times $\left(\sum_{i=1}^{k} n_i = N\right)$. The average (expected) value of the information obtained in the individual experiments will clearly be

$$\frac{-n_1 \log P(A_1) - n_2 \log P(A_2) - \ldots - n_k \log P(A_k)}{N}$$

If N is sufficiently large, then by the interpretation of probability n_i/N can be consid-

ered equal to $P(A_i)$; consequently, the above relation can be written in the form

$$-\sum_{i=1}^{k} P(A_i) \log P(A_i) \qquad [7.4]$$

which is identical with the entropy of the experiment. This means that the entropy of the experiment *is equal to the average value of the information contents of the individual outcomes of the experiment.*

Thus, the information content of the previously mentioned DNA molecule is $10^6 \log 4$.

In practice, instead of logarithms to the base 10 calculations are made with logarithms to the base 2. Consequently, an experiment has unit information content if its entropy calculated in a logarithmic system to the base 2 is 1. The unit information content obtained with logarithms to the base 2 is called one *bit*. One bit of information is obtained from an experiment which has two equally probable outcomes. The information content of the above DNA molecule will thus be 2×10^6 bits.

Figure 7.2 also demonstrates the information content of the experiment with two possible outcomes. This is indicated in bit units on the right-hand ordinate. The right- and left-hand ordinates differ only in their scales; the left-hand one gives the logarithms to the base 10, and the right-hand one those to the base 2, of the same numbers.

7.1.3. Examples on the utilization of information

1. The information content of macromolecules. If a *biological macromolecule* is known, there is no uncertainty left as to its structure. For this, information equal to the information content of the system has to be obtained. From [7.4], the information content of the base sequence of a DNA molecule consisting of 10^6 nucleotide bases is at most 2×10^6 bits. This is the amount of information to be collected by suitable physical and chemical methods to establish the base sequence of the DNA molecule, which determines the higher-order structure of the molecule too. The information content of the amino acid sequence of a protein molecule can be calculated similarly. Proteins are built up of 20 different amino acids, and thus the information content of the structure of a protein molecule consisting of, for example, 500 amino acids will be approximately 2×10^3 bits. This value is lower by three orders of magnitude than that found in the example of DNA, but the collection of even this information still presents a formidable task, because the determination of the sequence requires a number of experimental steps of the same order of magnitude (or only slightly less) as the number of bits of information to be collected.

Our example explains the well-known fact that research work succeeded first only in the sequencing of relatively small proteins and nucleic acids or nucleic acid fragments. One of the first structures determined was that of insulin, consisting of 51 amino acids, whose information content is merely approximately 200 bits. Recently the

base sequencing has considerably quickened up. The already revealed DNA segments consist of 10^4–10^5 nucleic acid bases, e.g. they contain 10,000–100,000 bits of information. For example a large number of small-size viral genomes and numerous important chromosome parts have been base sequenced with the new more efficient automatized methods.

2. The estimation of genetic information. As already mentioned, *genetic information* is stored by the nucleotide base sequence, and the protein-synthetizing system of the cells synthetizes the required protein in accordance with the base sequence. In the case of various viruses, the information necessary for the building-up of the virus proteins is stored in a single nucleic acid. Let us calculate for *how many different proteins* is information carried by the nucleic acid of a simple virus, bacteriophage MS2, consisting of 3.3×10^3 bases. The calculations are based on the following reasoning: the number of types of proteins that can be produced will be the number of times the information content of a molecule of average size to be found in the nucleic acid in question. Since an average protein (consisting of 500 amino acids) contains approximately 2×10^3 bits of information, phage MS2 with its approximately 6.6×10^3 bits allows the production of 3 types of proteins. Empirical results indicate that this calculation is correct. It is found in experience that for small viruses containing only a few bases (e.g. bacteriophage ΦX174), a given DNA part may contain information relating to two different proteins, i.e. the codes of the two proteins overlap each other in the nucleic acid. The above estimation of course does not consider this case; it yields only the *lower limit* of the possible number of proteins.

Information flow. The *capacity of an information communication channel* is characterized by the maximum information transmitted without noise per unit time. This is the information flow.

The capacity of a *single nerve fibre as an information channel* is less than 10^3 bits/s. This estimation is based on the fact that at most 10^3 action potential signals are transmitted through one fibre per second (the duration of an action potential is approximately 1 ms), and that the information is carried by the presence or absence of the action potential.[1] This alternative means a maximum of one bit of information. The above capacity value is obtained in this way. The capacity of a fibre bundle is given by the sum of the capacities of the individual fibres. The human organism receives about 10^{10} bits of information per second from the external world via the sense organs. This at first sight vast information flow is obtained by the following estimation. We consider only the information received by the eye. Each receptor cell (rod) ensuring twilight vision is found to be able to distinguish 32, i.e. 2^5 different brightness grades.

[1] The action potential in the present case is treated as a digital signal, which is not inconsistent with our treatment in section 6.4.1, where the same action potential was considered from a different aspect as an analogue signal.

Thus, on the appearance of a single image, one rod yields 5 bits of information. Since the human eye has to perceive one image for at least 1/16 s, and since the retina contains approximately 10^8 rods, the total information flow via the rods amounts to 10^{10} bits/s.

It may be mentioned (without going into details) that the cones contribute to this quantity with an information flow of approximately 10^9 bits/s.

Only ca. 100 bits/s of the information reaching the organism is consciously perceived, the rest being selected out. (Of this consciously perceived information 10 bits/s are stored for a short time in the central nervous system and 1 bit/s for a long time.) The selection may be achieved in various ways in a given organ. In the case of the eye, it begins with the fact that the number of *n. opticus* fibres is only of the order of 10^6, while the order of the number of receptor cells is 10^8. Since the channel capacity of one nerve fibre is less than 10^3 bits/s, the total capacity of the *n. opticus* is less than 10^9 bits/s.

7.2. Control

A considerable proportion of the processes in the various technical, biological, etc. systems occur in an ordered that is in a coordinated and controlled way. For instance, the adaptation of living organisms to their environment or the production in a factory is accomplished by control. This control is carried out either via conscious elements or without them (automatic control). In the examples considered both types occur, and in practice we are usually concerned with such cases.

The two basic functional elements of a control system are the controlled and the controlling units (controller). In the previously discussed examples both the controlled units and the control centres may be of many types. Thus, in the more highly developed living organisms the control centre for adaptation is the central nervous system, while in the factory the centre is the director, who may carry out his controlling of production with the aid of a computer centre.

Each type of control is based on the *transmission* of *information*. Information arrives at the control centre and departs from it. The arriving information may originate from sources outside the control system, but from inside the system itself too. However, information of the opposite direction always flows from the centre toward the controlled units.

The concept of control includes two types of processes: *simple control* (without feedback) and *control with feedback* or *regulation*. In control without feedback no information reaches the control centre from the controlled unit, that is the process does not react upon the control. In the case of regulation, on the other hand, there is feedback (negative feedback) and it is this feedback which enables the controlled system to serve the preset goal. In the following we shall deal with regulation in detail, since this process plays a fundamental role in the function of biological systems.

7.2.1. Regulation. The functional scheme of regulating systems

The structure and function of regulating systems. The task is to ensure that a given parameter of a system (e.g. temperature, pressure, the concentration of some chemical component) should always assume a prescribed value. We speak of *constant value control* if the value of the parameter has to be kept at the same level. For instance, the maintenance of the constant temperature of a thermostat is carried out by this type of regulation. The regulation of the body temperature, blood pressure, pH value, etc. of living organisms is of the same type. In *sequential control* the parameter value changes in accordance with the momentary actual requirement. As a biological example, repressive regulation of enzyme synthesis may be mentioned. In the case of a *time-schedule control,* the parameter changes according to a preset programme. A time-schedule control operates to ensure for example the heating rate of a thermostat with gradually increasing temperature, or the progress in the ontogenesis of a living organism.

The simplest control systems contain a single feedback system (these are the one-loop *regulating systems*). The general structure of these systems and the functions of the individual functional units (the functional block scheme) are depicted in Fig. 7.3. The arrows denote the connections between the units and the directions of information transmission. Communication is achieved through the input and output signals of the units. (In biological regulating systems, information is always carried by analogue signals.) The parameter characterizing the actual state of the regulated system is the *output signal,* while the *base* or *setting signal* gives the preset parameter value to be maintained by the system. Besides the *controller* and *controlled* (and *feedback*) units, every control system also contains a *comparator unit,* into which the base signal and the *feedback* or *control* signal (changing together with the signal of the controlled parameter) arrive. The comparator unit forms the difference of these two quantities[2] (negative feedback), and if it is different from zero the difference will

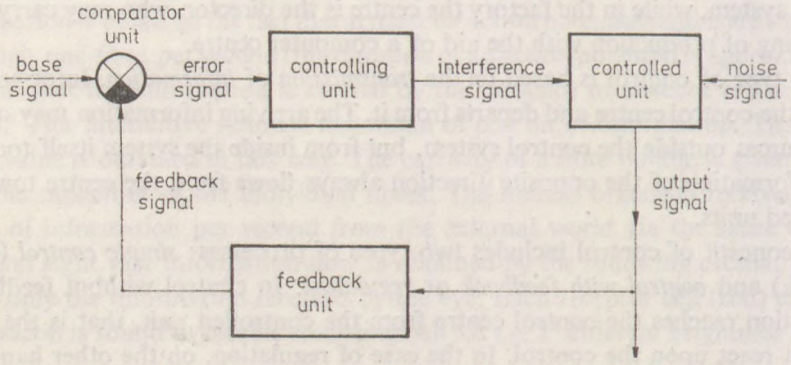

Fig. 7.3. Functional diagram of a simple regulating system

[2] By convention, the difference-forming function of the comparator unit is indicated in the drawing by blackening a quarter of the circle representing the unit.

pass back into the controller unit as an *error signal;* this in turn influences the controlled system by means of an *interference signal.* Besides this signal, other not negligible noise signals may affect the system. Naturally, the latter also influence the change of the output or feedback signals.

Examples of regulation. In this section we investigate the operation of some technical and biological regulating systems in order to demonstrate the actual performance of the functional units of the schematic diagram and the information transmitted and processed by them. Figure 7.4 depicts the functional diagram of an *electric thermostat* operating as a *constant value* regulating system. The actual temperature of the thermostat is measured with a thermocouple T, for instance. The thermovoltage (U) supplied by the thermocouple is the output signal, which is compared by feedback with the voltage U_0 corresponding to the required constant tem-

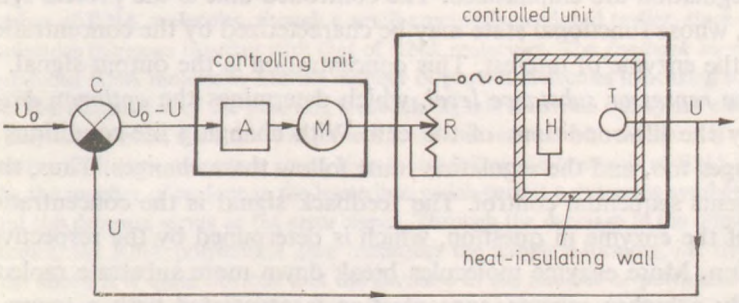

Fig. 7.4. Block diagram of an electric thermostat

perature. This function is performed by the comparator unit (C) which produces the difference of the two voltages ($U_0 - U$). After suitable amplification (A), the voltage difference operates an electric motor (M). (The amplifier and the electric motor together constitute the controller unit.) The rotation of the motor supplies the interference signal, which results in a displacement of the sliding contact of a variable resistor (R) connected in series with the thermostat heater (H). The motor turns in one or the other direction, depending on whether U is larger or smaller than U_0, and accordingly the contact slides to the required position. If $U > U_0$, the sliding contact moves to increase the circuit resistance, resulting in a decrease of the heating current and together with this the temperature of the thermostat. If $U < U_0$, however, the reverse process takes place. The sliding contact moves to and fro as long as the temperature deviates from the preset value.

Regulation as an organizing principle can be found at every level of the organism, from a molecular level up to sophisticated organ systems. Figure 7.5 depicts the functional diagram of a biological regulation at a molecular level, the regulation of enzyme synthesis. For the sake of better understanding, the more refined details of the

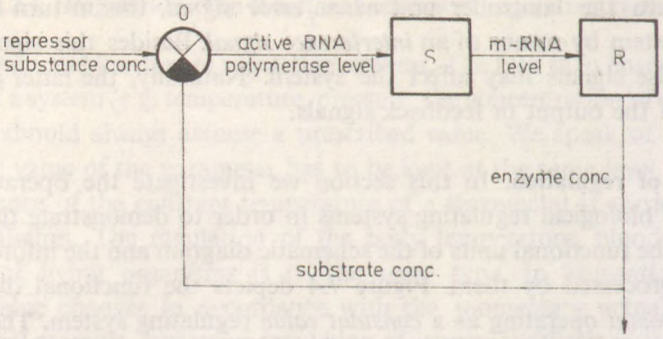

Fig. 7.5. Functional block diagram of enzyme synthesis

mechanism are omitted, and only the essential, general features important from the aspect of regulation are emphasized. The controlled unit is the protein-synthetizing system (*R*), whose functional state may be characterized by the concentration within the cell of the enzyme of interest. This concentration is the output signal. The base signal is the *repressor substance level,* which determines the *optimum enzyme level* required by the life-conditions of the cell. With changing life-conditions the base signal changes too, and the regulation must follow these changes. Thus, the present case represents sequential control. The feedback signal is the concentration of the substrate of the enzyme in question, which is determined by the respective enzyme concentration. More enzyme molecules break down more substrate molecules, and consequently a higher enzyme concentration is associated with a lower substrate level and vice versa. The feedback signal and the base signal, i.e. the substrate level and the repressor substance level, are compared in the following way. If the appropriate operator gene interacts with the repressor substance, no transcription takes place on the associated structure gene. However, if the substrate molecules inhibit the coupling of the repressor substance with the operator gene, DNA polymerase becomes activated and mRNA will be synthetized according to the structure gene base sequence. Thus, the operator gene (*O*) is the comparator unit and the error signal will be the active RNA polymerase level. It is clear that the structure gene (*S*) plays the role of the controller unit. The enzyme synthesis in the protein-synthetizing system (ribosome) proceeds in accordance with the mRNA copied from the structure gene. This means that the larger the error signal, the more mRNA will be synthetized, and as a consequence the synthesis of the enzyme in question will increase. The interference signal is the level of mRNA encoding the enzyme in question.

Another relatively simple example of biological regulation at a molecular level is the control mechanism acting in the *development of phage MS2.* This phage consists of a single RNA molecule surrounded by an envelope containing ca. 180 identical protein molecules. The regulation is related to the template and messenger functions of the RNA molecule. The former function means that in the course of development the single RNA molecule of the phage, having entered the bacterium cell,

serves as a template for the synthesis of further phage RNA molecules. A fundamental role in this process is played by the own RNA polymerase of the phage; this too is formed in the intrabacterial phage development and one gene of the phage contains the information for its base sequence. The RNA synthesis stops when no more RNA polymerase is synthetized on the basis of this gene (and the already existing molecules decompose). The messenger function of the RNA is the synthesis of the phage coat-protein; however, this becomes important only after the production of a sufficient number of RNA molecules. The genetic information necessary for the coat-protein synthesis is also supplied by the RNA, or more exactly by its appropriate gene.

In our example the *regulation* means that the phage RNA synthesis should stop at the right moment, while the production of coat-protein molecules in the required quantity and intensity is ensured. In the example, two different kinds of mechanism operate: one of them controls the template function and the other messenger function. However, it is sufficient to consider the template-forming system as a controlled system, since the messenger function is determined by the template function. The number of produced phage RNA molecules provides information about the state of the template-forming system; this is the output signal. Depending upon the physiological state of a bacterial cell, more or fewer phages may be synthetized. The number of phage RNA molecules is the *base signal.* An appreciable quantity of coat-protein can obviously be synthetized only after the formation of a sufficient number of RNA molecules, though a small amount is produced earlier, since the number of protein molecules increases together with that of RNA molecules. The *feedback signal* is not the number of produced RNA molecules, but the number of protein molecules increasing together with that of RNA. The *comparison* of the base and feedback signals is carried out by the starting region of the RNA polymerase gene, since the coat-protein molecules interact with the starting region of the RNA polymerase until their number is small, thereby inhibiting the synthesis of RNA polymerase. Consequently, the number of regions in the bacterium which permit polymerase synthesis gradually decreases, and this decrease serves as the error signal. Through the decrease in the number of polymerase molecules, the RNA polymerase gene influences the template-forming, i.e. the controlled unit. From the above it is quite obvious that the decrease in the number of polymerase molecules acts as an *interference signal,* and the RNA polymerase gene operates as a *controller unit.*

7.2.2. The study of regulating systems. Transition functions

The concept of dynamic analysis. Technical control systems are known in advance, since they are usually constructed from elements with well-known properties, and the elements are put together according to purpose. Under these circumstances it is understandable that the response of the system (output signal) to known external effects (input signals) can be determined quantitatively in advance. The reverse case is also conceivable, when conclusions concerning the effect produced on the system are drawn from the response. The situation is usually more difficult with biological control systems. The details of these systems are generally less well known, and the main task is to establish the structure of the system and the properties of its elements. On the other hand, the object of our investigation may be restricted to determining the response of the system to some external effect under given circumstances, or to determining what effect produced a given response. For instance, the consideration of the possible effects of some therapeutic intervention involves studying the possible responses of the biological system to a known external effect. In medical diagnostics,

on the other hand, from the change in a characteristic parameter of a biological system with well-known properties conclusions are drawn on the effect inducing this change.

In the study of a system the black box approach is a frequently used model. Any system may be treated by this model if its internal structure is not taken into account (or even if it is not known), and only the relations between the input and output effects (input and output signals) are investigated. This type of treatment is the *dynamic analysis*. It follows from the earlier discussion that the black box method, i.e. dynamic analysis, is quite frequently applied in the case of biological systems.

Example of dynamic analysis. Dynamic analysis is generally a rather complex method, since the testing of the system is carried out in various ways, using different input signals. Figure 7.6 shows some of the most frequently used testing signal forms. The horizontal axis of the diagram denotes time, while the ordinate relates to the interfering effect, that is to the change of some parameter ($f(t)$) of the control system. In diagram *a* the investigated parameter changes suddenly from one value to another, after which it keeps the new value for a prolonged period. In diagram *b* the parameter returns to its original value after a sudden rapid change. Diagram *c* demonstrates the testing when (disregarding a short initial period) the value of the parameter changes at a constant rate. The procedure outlined in diagram *a* is followed, for instance, in the study of a thermostat, if the temperature to be regulated is changed to a prescribed higher value. Case *b* is encountered when vessels stored in a thermostat are exchanged for colder ones. In case *c* the thermostat temperature increases according to a linear program.

The concept of the transition function. Let us discuss case *a* in some detail. Dynamic analysis (especially its mathematical treatment) is simplified if the rate of change of the parameter is so fast that the time interval ε may be regarded as zero ($\varepsilon \to 0$). In this case the parameter will change not as in Fig. 7.6*a*, but rather according to Fig. 7.7. Considering by definition a unit change, the function depicted in Fig. 7.7 is the *unit-step function*. In this case the dynamic analysis consists in studying the response

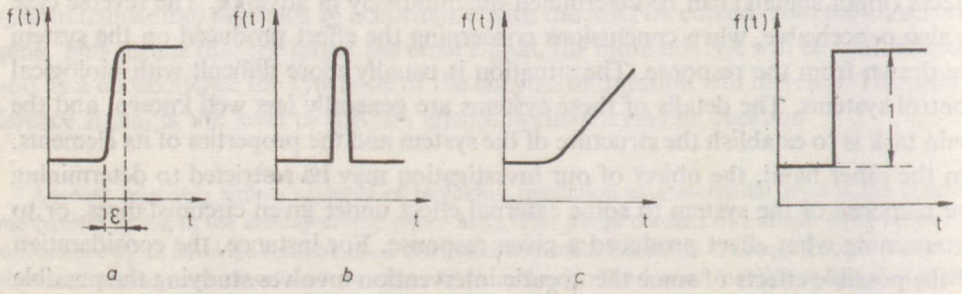

Fig. 7.6. Input signals of various shapes

Fig. 7.7. Unit-step function

of the system to the unit-step effect. The function describing the response, i.e. the time course of the output signal, is the *transition function*. Blood pressure regulation is a good example to demonstrate a biological transition function. On the basis of the black box model, this regulation may be discussed without describing the functional structure of the system. The object of the actual investigation is the response developed by the organism when, for instance, a person suddenly stands up from a lying position. The positional change corresponds to the unit step. The response is a sudden decrease of the arterial mean pressure (approximately 13 kPa), which subsequently usually returns to its original value within a few seconds. The return process may follow different time courses; the three most characteristic types are depicted in Fig. 7.8. Diagram *a* shows an aperiodically damped response, *b* a periodically damped one, and *c* an undamped response. This latter case is always associated with a defective regulation. It should be noted that in the case of the voltage clamp technique discussed in section 6.2.2 the sudden setting of the membrane voltage corresponds to the unit-step signal, and the separated ionic fluxes to the aperiodically damped response of the system.

The response of the system, i.e. the transition function, consists of two parts. One part changes with time (this is the transient part), whereas the other remains constant (this is the stationary part). The transient part is determined by the internal properties of the system, and the stationary part may be influenced not only by the properties of the system, but by external effects too. In our example both parts can be observed in Fig. 7.8*a*: the transient period lasts approximately until the 30th second, after which the stationary part follows. (The parameter value characterizing the sta-

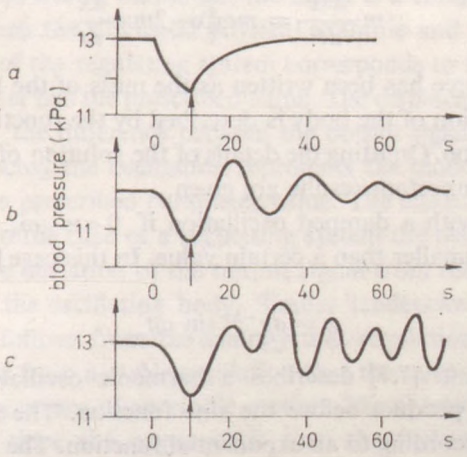

Fig. 7.8. Variation of the output signal of the blood-pressure regulating system in time (transition function), due to a sudden change in state (unit-step function). The diagram depicts the results of actual measurements
The zero point on the time axis corresponds to the time of the position change; the response of the control system begins at the time indicated by the arrow

tionary period agrees in this case with that before the unit step.) Diagram *b* shows only the transient part, the stationary part simply being its continuation. In diagram *c* the stationary part is missing, and the control system operates defectively.

The transient part of transition functions can be well illustrated by a simple physical analogy. Let us consider the motion of a body fastened to a spiral spring. (The mass of the spring can be neglected with respect to the mass of the body.) Let us displace the body in the vertical direction from its equilibrium position and then leave it alone. It is found in experience that the motion of the body is a damped oscillation. The damping may be so strong that the displaced body will not move in the other direction beyond its equilibrium position, but approaches the equilibrium position from the direction in which it was originally displaced.

In the interpretation of this phenomenon two forces are considered. The first is the elastic force (X_e) inducing the motion, while the second is the frictional force (X_f) impeding the motion. The elastic force may be taken as proportional to the displacement (x) and its direction is opposite to that of the displacement. The frictional force, on the other hand, may be taken as proportional to the velocity (dx/dt) of the motion, this force also being opposite in direction to the displacement. The following relations hold

$$X_e = -m\omega_0^2 x \quad \text{and} \quad X_f = -2m\varkappa \frac{dx}{dt} \qquad [7.5]$$

where m denotes the mass of the body, and ω_0 and $\varkappa$ are constants characterizing the elastic and frictional force, respectively. (ω_0 is the angular frequency of frictionless vibration.) The resultant force is given by the following differential equation

$$m\frac{d^2x}{dt^2} = m\omega_0^2 x - 2m\varkappa \frac{dx}{dt} \qquad [7.6]$$

where the resultant force has been written as the mass of the body multiplied by its acceleration. The motion of the body is described by the functions $x(t)$ which satisfy the differential equation. Omitting the details of the solution of the differential equation, only the more important results are given.

The body moves with a damped oscillation if $0<\varkappa<\omega_0$, i.e. if the friction is larger than zero, but smaller than a certain value. In this case [7.6] is satisfied by the relation

$$x = ae^{-\varkappa t} \sin \omega t \qquad [7.7]$$

where a is a constant. [7.7] describes a harmonic oscillation whose amplitude is determined by the product before the sine function. The value of this product decreases with time according to an exponential function. The rate of decrease is the faster, the larger the value of $\varkappa$, i.e. the larger the friction (Figs 7.9*b–c*). If $\varkappa=0$, i.e. if there is no friction, the harmonic oscillation of the body is not damped (Fig. 7.9*a*). The angular frequency ω of the damped oscillation is smaller than the frequency ω_0 of the undamped oscillation: $\omega^2=\omega_0^2-\varkappa^2$.

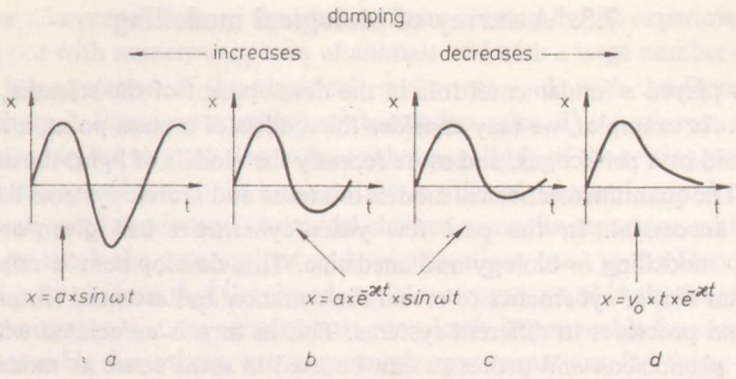

Fig. 7.9. The motion of an oscillating body with various dampings

The body does not oscillate, but moves aperiodically if $\varkappa \geqq \omega_0$, i.e. if the friction is larger than or equal to a certain value. First let us consider the case when $\varkappa = \omega_0$; this is the *aperiodic limiting case*. Let v_0 denote the velocity with which the body at rest is pushed at time $t=0$. After being pushed and subsequently left alone, the motion of the body is described by the relation

$$x = v_0 t e^{-\varkappa t} \qquad [7.8]$$

According to [7.8] the displacement first increases, then reaches a maximum and subsequently decreases; the body gradually approaches its equilibrium position (which in principle is reached after an infinitely long time; Fig. 7.9*d*). The particulars of the case $\varkappa > \omega_0$ will not be discussed; it should be mentioned only that the approach to the equilibrium position will be the slower, the larger is $\varkappa$ compared to ω_0.

The analogy between the discussed physical example and the regulating system is obvious. The state of the regulating system corresponds to the body at rest when the controlled parameter has the prescribed value. The displacement of the oscillating body is analogous to the difference between the output signal and the base signal. The elastic force inducing the oscillation represents the tendency of the regulating system to maintain the prescribed parameter value. The elastic force is proportional to the displacement; in the case of a regulating system the restoring tendency is the stronger, the larger the deviation of the output signal from the base signal. Friction damps the motion of the oscillating body. Similar tendencies are operative in the regulating systems. It follows from the analogy that regulation occurs only in their presence. It is expected from a *stable* regulation that the output signal will approach the prescribed value by means of aperiodic motion. The aperiodic limiting case is the most favourable one, since the approach is then the fastest. Figure 7.9 demonstrates the relation between the stability of regulation and the transient part of the transition functions as well.

7.3. A survey of biological modelling

Models played a fundamental role in the development of the sciences, especially of physics. As examples, we may consider the models of a mass point, a rigid body, an ideal fluid or a perfect gas, and more recently the models of light, the atom or the molecule. The quantum mechanical models of atoms and atomic systems have proved extremely successful. In the past few years cybernetics has given considerable impetus to modelling in biology and medicine. This development is related to the fundamental aim of cybernetics to reveal the common and essential features of phenomena and processes in different systems. This in turn is associated with the fact that these phenomena and processes can be used in some sense as models of each other. Moreover, cybernetics has not only contributed to the elucidation of the theoretical bases of modelling and made its application a conscious one but has also widened the possibilities of modelling (cf. section 7.4).

Modelling in biology and medicine is nowadays a useful tool permitting the clarification, understanding and interpretation of various phenomena and processes, from a molecular level up to the living organism. Thus, a model is constructed when the repressive regulation of enzyme synthesis is interpreted in terms of a single-loop control circuit. Some properties of the membranes of excitable cells can be modelled by electric circuits, and a model is made whenever the physician tries to produce some human clinical pattern in experimental animals, or when the efficiency of a new drug or inoculation is tested in animal experiments. Since the role and importance of modelling have increased as a result, a critical appraisal of modelling appears worthwhile.

Every model is to some degree only an approximation to reality, and therefore it is always necessary rigorously to test the conclusions drawn from it. A model usually emphasizes only certain features of the investigated phenomena and processes; it does not give any (or only a slight) possibility for the study of others. Of the important features of modelling, the following may be stressed:

— modelling creates connections between various groups of phenomena;

— model studies can be carried out under conditions so extreme that biological systems cannot tolerate them without functional damage;

— models which can be formulated mathematically are especially valuable, since these models may be compared quantitatively with real systems.

The actual steps of modelling are demonstrated on a well-known example, the resting potential.

The acquiring of knowledge begins with the *perception* and *observation* of the phenomenon (process). In the case of the resting potential it has been observed that if a damaged frog muscle is brought into contact with another muscle, it induces the contraction of the second muscle. A very great number of systematic *experiments* were necessary to prove the existence of a potential difference between the intra- and the extracellular space. Experiments performed under different conditions led to the

quantitative characterization of the phenomenon. (Naturally the experiments had to be carried out with numerous species of animals and with a large number of tissues.) The first interpretation of the empirical results was made with the Donnan model (the first theory), based on equilibrium thermodynamics. The *mathematical description of this model* led to a relation between the magnitude of the resting potential and the concentrations of the mobile ions on the two sides of the membrane. A *comparison of the experimental results* and the model showed some discrepancies, which made it necessary to check the assumptions and simplifications used in the Donnan model. It became quite obvious that the closed model system assumed in the Donnan model, and the assumption that the membrane is completely impermeable for some ions and wholly permeable for others, are very rough approximations for living cells. This finding and new experimental results led to an *improvement and modification of the model*. The new model, that of Hodgkin–Huxley–Katz, which was based on the thermodynamics of transport processes, shows better agreement with the real system. However, this interpretation too is only an intermediate *step* in the course of acquiring knowledge, and the problem of the resting potential can still be regarded as settled, as has been demonstrated in section 6.2.2.

Figure 7.10 summarizes the above steps. The diagram demonstrates rather well the cyclic process of acquiring knowledge, by which we proceed towards a continuously improving understanding of a phenomenon.

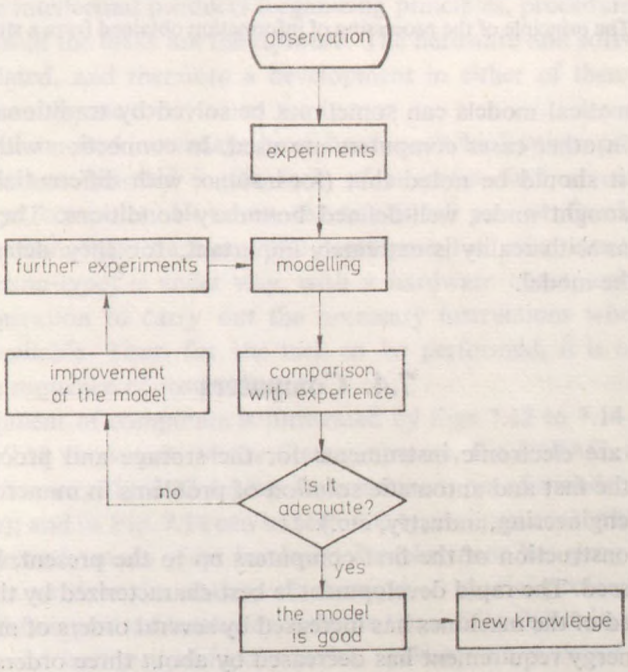

Fig. 7.10. Block diagram of the acquisition of scientific knowledge

The act of modelling is quite frequently a complex task, which is demonstrated in Fig. 7.11 in some detail. The investigated system is in most cases modelled on some *physical or physicochemical analogy,* which always means considerable abstraction. In the course of this modelling process, numerous properties of the actual system are discarded and only the essential details are emphasized. In the case of the usually rather complex biological phenomena, this method involves considerable simplification and omission; however, this is consciously accepted for the sake of clarity. Mostly, physical models are in fact constructed, but in some cases they are used only to give a mathematical description of the essential features of the real system (e.g. by means of differential equations). In certain cases the studied system leads directly to the mathematical model as shown in Fig. 7.11.

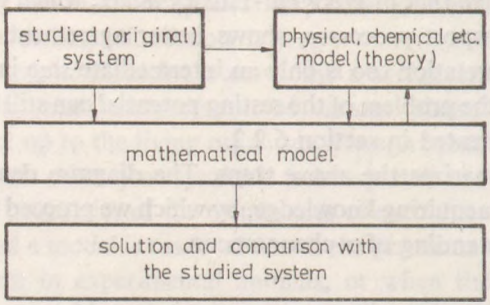

Fig. 7.11. The principle of the processing of information obtained from a studied system

The mathematical models can sometimes be solved by traditional mathematical methods, but in other cases computers are used. In connection with the mathematical methods it should be noted that (for instance with differential equations) the solutions are sought under well-defined boundary conditions. The comparison of these conditions with reality is extremely important, for they determine the limits of validity of the model.

7.4. Computers

Computers are electronic instruments for the storage and processing of data; they serve for the fast and automatic solution of problems in numerous fields of life (e.g. research, engineering, industry, etc.).

From the construction of the first computers up to the present, less than half a century has passed. The rapid development is best characterized by the facts that the computing speed of the machines has increased by several orders of magnitude, while their electric energy requirement has decreased by about three orders of magnitude. Both facts are related to the enormous progress in the development of the electronic

devices applied in computer design, from electron tubes through transistors to solid-state circuits.

In the past decade computers have become widely applied in both medical research and practice, and the scope of their use is constantly increasing. Nowadays computers are used not only for the processing, storage and retrieval of a very high number of measurement data, but for the automatization of experimental and measuring processes as well. These possibilities are utilized in medical research and in the automatization of clinical chemical laboratory diagnostics too. In the most modern, high-efficiency medical diagnostic procedures, the actual measurements, the processing, the storage and the imaging of the results are all carried out by computer. Computers constructed for special tasks are applied in the γ-camera (section 2.18) in computer-assisted tomography (section 2.12) and in NMR-tomography (section 3.5.1). Today, even medical therapy cannot exist without computers (e.g. the planning of irradiation is performed with computers) and it is to be expected that computers will play further important roles in the automatic performance of various types of surgical intervention (e.g. stereotactic surgery).

7.4.1. The basic structure and operation of computers

The assembly of technical components comprising a computer is called the *hardware,* while the intellectual products (organizing principles, procedures) ensuring the accomplishment of the tasks are the *software*. The hardware and software are always closely interrelated, and therefore a development in either of them influences the progress of the other one too.

Most computers used nowadays have hardware which solves problems by consecutive instructions encoded in the form of numbers. Such a computer is called a *Neumann-type* computer. However, the trends of present development extend beyond the Neumann-type machines. The development of other types of automatons (i.e. non-Neumann-type) is under way, with a hardware construction that renders possible an operation to carry out the necessary instructions when all the data required are available. Thus, for the task to be performed, it is not necessary to have a definite sequence of instructions.

The development of computers is illustrated by Figs 7.12 to 7.14 (in the Supplement). Figure 7.12 shows one of the first computers, the ENIAC, already of only historical significance; Fig. 7.13 depicts the machine room of a modern large computer (main frame); and in Fig. 7.14 can be seen one type of personal or home computers, these having recently made great headway. Development has progressed from the large computers used in the sixties, which required special professional personnel performing highly organized work, towards the "user-friendly" microcomputers, not requiring a special background in computing techniques: ***personal or home computers.***

The present devices in computer and communication techniques point to further perspectives. The connection of large, medium and microcomputers to form a computing and telecommunication network may render it possible for both communities and individuals to benefit from the direct and fast use of a vast amount of information. This will serve not only the advance in science and technology, but social progress as well.

The block diagram of a computer is shown in Fig. 7.15. The peripherals serve for information transfer between the computer and the external world, in both directions (input and output). Data and instructions may be entered into the computer via the peripherals, and the results of tasks performed by the computer also appear in the peripherals. Depending on the operating speed, *slow* and *fast* peripherals can be distinguished. In the operation of slow peripherals, mechanical parts with a greater inertia play the dominant role, as indicated by the name. Examples of slow peripherals are paper-tape punchers, punched-tape readers, card punchers and readers, line printers and XY plotters (graphical recorders). In the operation of fast peripherals, the electronic parts predominate. Fast peripherals are magnetic tape and disc drives and the *visual display*. This latter shows on its screen the information entered into the computer in the form of alphanumeric and special characters, thereby making possible the control and correction of data and/or instructions; the results of calculations carried out by the computer also appear on the screen, in the form of numbers, words or diagrams. The display is usually a special cathode-ray tube terminal or monitor with a keyboard, but in the case of personal computers an ordinary TV set may also be used as display.

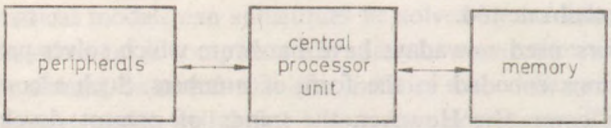

Fig. 7.15. Block diagram of a digital computer

As input and output peripherals of large computers working in the multiprogramming or time-sharing mode, the *terminals* should be mentioned. Their task is to enter data into the central computers and to retrieve (display or record) results computed in the centre. The terminals and the central computers are connected by telephone, telex or other special transmission lines functioning via satellites, for instance. For the connection between the terminal and the computing centre, the conversion of signals is necessary, i.e. the conversion of digital signals into signals suitable for transmission through telecommunication lines, and vice versa. This is the function of the "modem" unit (modulator–demodulator). Some personal computers are also suitable, or supplemented with a modem unit may be made suitable for use as terminals, i.e. to be joined to large central computers.

The *central processor* unit (CPU) executes electronically the basic arithmetic and logical operations appearing in it in the form of numbers, and supervises the function of the whole system. In the course of development the speed of operation has increased considerably; at present a processor carries out 10^8–10^9 elementary operations per second.

The great development of solid-state circuits and the lowering of their price made possible the construction and great proliferation of *microprocessors* (cf. section 5.6.4) serving as processors of computers. A microprocessor may contain several ten thousands of transistors on a semiconductor chip in one single integrated circuit. The CPU of personal computers is a microprocessor.

An essential part of the computer is the *memory unit* (Fig. 7.15). Its function is the storage of data and instructions necessary for the direct execution of tasks, and it is therefore often called the *operative memory (primary memory)*. One part of the operative memory is the Read Only Memory or ROM, while the other is the Random Access Memory or RAM. The *ROM part* is the fixed memory, where the information is stored permanently. Here the information storage may be carried out either optically or electronically. As examples of permanent optical information storage, the photographs and holograms might be mentioned; examples of electronic storage are solid-state circuits. The *RAM part* of the memory is called read/write memory too. The name indicates the function: after the writing-in, the information is readable as long and as many times as necessary, and in place of the stored information new information can be written in if required. The information stored in the RAM is usually lost after electric network power failures and after switching off the machine.

In chips applied as semiconductor memories it is possible to store a very large quantity of information in a very small volume (a few tenths of a cubic centimetre). At present 64 kilobits of information may be stored on a chip of average capacity, and the development in this field aims at a further increase in the storage capacity.

However, development can be conceived outside the field of solid-state circuits too; work is now continuing on the understanding and utilization of information storage at a "molecular" level, on the basis of other principles. In this respect mention may be made of the molecular information storage accomplished in nucleic acids in Nature: the average information content of 50 thousand base pairs (cf. section 7.1.3) is about 100 kilobits, i.e. approximately the same as that of semiconductor chips. However, this quantity of information, in contrast to semiconductor memories, occupies a volume of only ca. 10^{-16} cm^3.

Among the peripheral devices, the carriers of information constituting the *background memory* or *external memory* have already been mentioned. From this type of memory the information may be transferred to the operative memory. Possibilities are magnetic tapes (casettes) or magnetic discs. On these the information is stored in accordance with the direction of magnetization. The write-in of information is the setting of the direction of magnetization, and the read-out is the sensing of this

direction. It is characteristic of the background memory that the information remains unchanged after the switching-off of the machine.

The most recent stage in the development of computers was the appearance of *personal or home computers*. They began to be marketed somewhat less than 10 years ago, and may be considered an important step in the formation of a direct contact between man and computer. The great advantage of personal computers lies in their flexibility and wide range of applications to suit individual requirements. They can be used in office and household administration, or for the fast retrieval of information stored in a central health data bank, for instance, which may prove life-saving in many cases. They also find use in schools, for the teaching of elementary arithmetic, and are likewise a source of entertainment being ideal for the playing of games. The functioning of personal computers is based on microprocessors, which are becoming cheaper and cheaper, and it may therefore be expected that personal computers will be found in general everyday use in the near future. A very great advantage is the fact that no previous training in computing techniques is necessary for their use; a background memory can be added to them (e.g. a tape recorder), and thus their information-storing capacity may be increased arbitrarily.

The hardware, or more exactly the processor and memory of a computer, determines its information-storing and processing capacities. However, the user does not come into direct contact with these parts of a computer, since a program-hierarchy intervenes between the user and the hardware. The user prepares the description of the task to be solved, the program, in a programming language. The programming language is a set of instructions which can be processed by the computer. For the processing, the machine uses built-in programs and with their aid breaks down the task given by the application program into the elementary instructions used by the processor.

The programming languages differ in the *level* to which the user (the application program) has to break down the task so that it is suitable for the machine to process. The lowest-level programming languages work on an *algorithmic level*. Their application requires a description of the whole process of the solution in the form of unique instructions. The best-known languages at this level are ALGOL, FORTRAN, COBOL, PL–1, PASCAL and BASIC. The latter is often used in personal computers. Programs prepared in the next higher, *logical-level* languages require only the definition (determination) of the task, but the method of solution (its algorithm) need not be given as this is performed by the machine. Such languages are the languages of artificial intelligence, e.g. PROLOG. The *highest-level* languages are suitable for the solution of not well-defined problems as well. In these languages only an approximate statement of the problem is necessary and the correct solution is obtained via an interactive dialogue between the computer and the user. These highest-level languages are currently in the stage of development, but their future importance in medicine can be predicted, mainly in diagnostics.

7.4.2. On the medical application of computers

As has been already pointed out, computers have been used in an increasing number of fields in the last ten years. In the following some medical applications will be discussed, in which the computers have attained important functions. It is expected that this importance will become even more influential.

(a) One field of application is represented by the so-called *intelligent measuring devices*. In more simple cases these devices include built-in microcomputers which simplify their operation by displaying the measured data properly processed and already arranged in groups. As an example let us consider an up-to-date spectrophotometer (cf. section 3.3). The simple operation of this apparatus is realized by an automatic change of the wavelength of the measuring light (e.g. in steps per 2 nanometers), moreover by the analogous or digital processing which produces the spectrum and its first and second (eventually even higher) derivatives. — In advanced equipments the computer systematically controls the state of the apparatus, eliminates certain defects and furnishes information for trouble-shooting and repair. In the case of spectrophotometer the control pertains to the intensity of the measuring and reference light and provides a constant intensity. This latter operation is realized by including special regulating circuits into the system. This built-in converter immediately transforms the analogous signals into digital ones which can be stored. For a more extensive processing frequently more efficient computers are required than those which can be built in into the equipment. Recently the development of direct contacts between the measuring instruments and some big computers has become possible. Functionally this contact may be compared with the communication between the eye and the brain: the eye, which corresponds to the intelligent measuring instrument, takes up, summarizes and processes the optical information (cf. section 7.1.3). This way the overload of the centre, i.e. the brain, can be avoided.

A whole series of intelligent equipments are operating in clinical laboratories. The substances to be measured by these instruments are provided by computers, which also perform the control, and co-ordination of operations.

(b) *Data recording* is the traditional use of computers with large, secondary memories which enable the handling, storing and systematization of a very large number of data. Also data selection according to any desired system has become possible. The required data are obtained within a few seconds either on the display or by print-out. The data stored in the memories can be multiplied, changed or deleted.

Medical data recording extensively uses these possibilities, here only briefly sketched. The computerized data handling of the filing system of a medical district as well as of larger institutions (hospitals, clinics, outpatients' departments, surgeries, etc.), further on urban, county and even central national data banks work this way. In this latter case the medical data of every citizen (e.g. birth, sex, children's diseases, protecting inoculations, vaccinations, diagnostical results, therapy) are stored in a single computer or in a system of several interconnected computers. The required

data may be immediately obtained in any part of the country by terminals connected to the national computer network. This enables the completion of the data with recent diagnostical, therapeutical results whenever it appears to be necessary.

The connection of the intelligent measuring instruments and the high-efficiency computers enabled the development of *medical diagnostic equipments* of great importance such as the gamma-camera (section 2.18), CT (section 2.12), MRI (section 3.5.1) PET (section 2.18), or ultrasound echography (section 5.4.2).

(c) The more recent medical application of computers consists of the *diagnostic use* of *expert systems*. These systems summarize every experience available about any disease at a given time, and help the physician to make his diagnosis with the aid of these data. The expert systems contain rules based on the most up-to-date knowledge availabe. With these and the use of the patient's data (e.g. symptoms, results of laboratory analysis) the computer gives its diagnosis, or in more complicated, rather frequently occurring cases gives diagnostic possibilities together with the pertaining probabilities. Naturally the expert system cannot substitute the physician and plays only a consulting role, so that the physician and the computer are in a dialogue with each other. In the course of this dialogue the physician may give information and instructions, besides asking questions, and may also direct the operation of the computer by emphasizing, weighting the data and by entering new aspects. The computer gives advices, asks for new information and, if asked, explains its decisions. In more recent versions the computers may acquire also "learning" abilities; this enables the modification of the stored rules or the entering of new rules obtained from actual case studies and experiences.

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8. TABLES

Table 8.1

The International System of Units
(Système International d'Unités; notation: SI)
Base Units

Base quantity	Base unit	
	name	symbol
Length	metre	m
Mass	kilogram	kg
Time	second	s
Electric current intensity	ampere	A
Thermodynamic temperature	kelvin	K
Amount of substance	mole	mol
Luminous intensity	candela	cd

Table 8.2

Derived SI units with special names

Quantity	SI unit		
	name	symbol	expressed with other SI units
Plane angle	radian	rad*	$m\ m^{-1}$
Solid angle	steradian	sr	$m^2\ m^{-2}$
Frequency	hertz	Hz	s^{-1}
Force	newton	N	$J\ m^{-1}$
Pressure	pascal	Pa	$N\ m^{-2}$
Energy, work, quantity of heat	joule	J	N m
Power	watt	W	$J\ s^{-1}$
Electric charge	coulomb	C	A s
Electric potential, electric voltage	volt	V	$W\ A^{-1}$
Electric capacitance	farad	F	$C\ V^{-1}$
Electric resistance	ohm	Ω	$V\ A^{-1}$

Table 8.2 (continued)

Quantity	SI unit		
	name	symbol	expressed with other SI units
Electric conductance	siemens	S	$A\ V^{-1}$
Magnetic flux	weber	Wb	V s
Magnetic flux density	tesla	T	$Wb\ m^{-1}$
Inductance	henry	H	$Wb\ A^{-2}$
Luminous flux	lumen	lm	cd sr
Illuminance	lux	lx	$lm\ m^{-2}$
Activity of a radioactive source	becquerel	Bq	s^{-1}
Absorbed dose	gray	Gy	$J\ kg^{-1}$
Dose-equivalent	sievert	Sv	$J\ kg^{-1}$

* should not be mistaken for the traditional unit of the absorbed dose, also denoted by rad

Table 8.3

SI prefixes

Prefix		Multiplier
name	symbol	
exa	E	10^{18}
peta	P	10^{15}
tera	T	10^{12}
giga	G	10^{9}
mega	M	10^{6}
kilo	k	10^{3}
hecto	h	10^{2}
deca	da	10
deci	d	10^{-1}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}
atto	a	10^{-18}

Table 8.4

Supplement on the use of measurement units

1. The plane angle may be also expressed in terms of the *degree* (denoted by °), its 60th part the *minute* (denoted by ′) and its 3600th part the *second* (denoted by ″)

$$1° = \frac{\pi}{180}\ \text{rad}$$

2. Time units used without restrictions are the *minute* (denoted by min), the *hour* (denoted by h), the *day* (denoted by d) and the calendar units, i.e. the week, month and year

$$1\ \text{d} = 24\ \text{h} = 1440\ \text{min} = 86\,400\ \text{s}$$

3. In atomic and nuclear physics the *atomic mass unit* (denoted by u) may be used, which is one-twelfth of the mass of the carbon 12 atom

$$1\ \text{u} = 1.66 \cdot 10^{-27}\ \text{kg}$$

4. The energy unit *watt-hour* (notation: Wh) can be used without restriction

$$1\ \text{Wh} = 3600\ \text{J}$$

The energy unit which may be used in atomic and nuclear physics is the *electron-volt* (denoted by eV)

$$1\ \text{eV} = 1.6 \cdot 10^{-19}\ \text{J}$$

5. The temperature unit *Celsius degree* (denoted by °C) may be used without any restriction. The temperature 0 °C is equal to 273.16 K (Kelvin degrees). The Celsius degree as a temperature difference is equal to the kelvin.
6. In the case of the amount of substance the elementary entity must be specified: atom, molecule, ion, electron, etc.
7. The SI unit of the concentration of a substance is mol/m^3: the quantity of the component in question in a mixture of 1 m^3 is 1 mol.
8. The SI unit of the *molality* is mol/kg: in a solvent of 1 kg the quantity of the component in question is 1 mol.
9. For the determination of the pressure of a fluid and of a gas, besides the pascal the *bar* may be used (denoted by bar)

$$1\ \text{bar} = 10^5\ \text{Pa}$$

Table 8.5

Interconversion of traditional and SI units

Table 8.5.1

Force units

	dyne	newton	pond	kilopond
dyne	1	10^{-5}	$1.02 \cdot 10^{-3}$	$1.02 \cdot 10^{-6}$
newton	10^5	1	$1.02 \cdot 10^2$	$1.02 \cdot 10^{-1}$
pond	981	$9.81 \cdot 10^{-3}$	1	10^{-3}
kilopond	$9.81 \cdot 10^5$	9.81	10^3	1

Table 8.5.2

Energy units

	erg	joule	metre-kilopond	kilowatt-hour	litre-atm	calorie	electron-volt
erg	1	10^{-7}	$1.02 \cdot 10^{-8}$	$2.78 \cdot 10^{-14}$	$9.87 \cdot 10^{-10}$	$2.39 \cdot 10^{-8}$	$0.624 \cdot 10^{12}$
joule	10^{7}	1	0.102	$2.78 \cdot 10^{-7}$	$9.87 \cdot 10^{-3}$	0.239	$0.624 \cdot 10^{19}$
metre-kilopond	$9.81 \cdot 10^{7}$	9.81	1	$2.72 \cdot 10^{-6}$	$9.68 \cdot 10^{-2}$	2.34	$0.612 \cdot 10^{20}$
kilowatt-hour	$3.6 \cdot 10^{13}$	$3.6 \cdot 10^{6}$	$3.67 \cdot 10^{5}$	1	$3.55 \cdot 10^{4}$	$8.6 \cdot 10^{5}$	$2.25 \cdot 10^{25}$
litre-atm	$1.013 \cdot 10^{9}$	$1.013 \cdot 10^{2}$	10.33	$2.815 \cdot 10^{-5}$	1	24.22	$0.633 \cdot 10^{21}$
calorie	$4.187 \cdot 10^{7}$	4.187	0.427	$1.16 \cdot 10^{-6}$	$4.13 \cdot 10^{-2}$	1	$2.63 \cdot 10^{19}$
electron-volt	$1.6 \cdot 10^{-12}$	$1.6 \cdot 10^{-19}$	$1.63 \cdot 10^{-20}$	$4.45 \cdot 10^{-26}$	$1.58 \cdot 10^{-21}$	$3.83 \cdot 10^{-20}$	1

Table 8.5.3

Pressure units

	dyn cm^{-2}	pascal (Pa)	p cm^{-2}	tech. atm. (at)	phys. atm. (atm.)	torr (mm Hg)	bar
dyn cm^{-2}	1	10^{-1}	$1.02 \cdot 10^{-3}$	$1.02 \cdot 10^{-6}$	$9.87 \cdot 10^{-7}$	$7.5 \cdot 10^{-4}$	10^{-6}
pascal (Pa)	10	1	$1.02 \cdot 10^{-2}$	$1.02 \cdot 10^{-5}$	$9.87 \cdot 10^{-6}$	$7.5 \cdot 10^{-3}$	10^{-5}
p cm^{-2}	981	98.1	1	10^{-3}	9.68	0.736	$9.81 \cdot 10^{-4}$
tech. atm. (at)	$9.81 \cdot 10^{5}$	$9.81 \cdot 10^{4}$	10^{3}	1	$9.68 \cdot 10^{-1}$	736	0.981
phys. atm. (atm)	$1.013 \cdot 10^{6}$	$1.013 \cdot 10^{5}$	1033.23	1.03323	1	760	1.01325
torr (mm Hg)	1333	133.3	1.36	$1.36 \cdot 10^{-3}$	$1.32 \cdot 10^{-3}$	1	$1.333 \cdot 10^{-3}$
bar	10^{6}	10^{5}	$1.02 \cdot 10^{3}$	1.02	$9.87 \cdot 10^{-1}$	750	1

Table 8.6

Some important material constants (solids)

Material	Density at 20 °C	Linear thermal expansion coefficient*	Specific heat**	Melting point	Heat of fusion	Tensile modulus	Tensile strength
	$\frac{kg}{dm^3}$	$\frac{1}{K}$	$\frac{kJ}{kg\,K}$	°C	$\frac{kJ}{kg}$	$\frac{kN}{mm^2}$	$\frac{N}{mm^2}$
		0.0000					
Aluminium	2.7	24	0.9	660	398	70	60–160
Brass	≈8.5	19	0.39	≈920		105	330–530
Bronze	8.8	16	0.39	1083	209	126	400–450
Glass	2.4–2.8	03–10	0.75–0.80			50–80	30–90
Gold	19.3	14	0.13	1064	63	80	110–130
Iron and steel types	7.7–8.9	09–12	0.46–0.54	1200–1540		115–195	140–350
Lead	11.3	29	0.13	328	25	16	15–18
Platinum	21.5	09	0.13	1772	101	160	130–200
Quartz	2.7	006	0.73	≈1700		60	800
Silver	10.5	19	0.24	961	105	76	140–380
Tungsten	19.3	05	0.13	3410	192	360	1000–4000

*Relative change of length for 1 K variation of temperature; the volume expansion coefficient is about three times this value

**At ordinary temperature and pressure

Table 8.7

Some important material constants (fluids)

Materials	Density at 20 °C	Surface tension*	Viscosity at 20 °C	Volume expansion coefficient*	Specific heat*	Melting point	Heat of fusion	Boiling point	Critical temperature
	$\frac{kg}{dm^3}$	$\frac{mJ}{m^2}$	mPa s	$\frac{1}{K}$	$\frac{kJ}{kg\ K}$	°C	$\frac{kJ}{kg}$	°C	°C
Acetic acid	1.05	28	1.22	0.00 107	1.97	16.6	192	117.9	322
Acetone	0.79	24	0.32	149	2.22	−94.8	98	56.1	236
Benzene	0.88	29	0.65	124	1.72	5.5	128	80.1	289
Chloroform	1.49	27	0.57	127	0.96	−63.5	75	≈61	262
Diethyl ether	0.71	17	0.24	166	2.26	−116.3	98	34.6	194
Ethanol	0.79	23	1.19	112	2.43	−117.3	108	78.5	244
Glycerol	1.26	63	1.49	050	2.39	20	201	290	452
Mercury	13.55	≈476	1.55	018	0.13	−38.9	12	357	1460
Olive oil	0.91	33	84	072	1.97				
Water common	0.998	73	1.0	≈02	4.18	0.0	334	100	374.2
Water heavy	1.105	68	1.25	23	4.21	3.8	318	101.4	371.5

* At common temperature and pressure

Table 8.8

Some important material constants (gases)

Gases	Density* (0 °C)	Viscosity* (20 °C)	Boiling point*	Critical	
				temperature	pressure
	$\frac{kg}{m^3}$	mPa s	°C	°C	MPa
Air	1.29	18.2	−193	−140.6	3.8
Carbon dioxide	1.98	14.8	− 78.5**	31	7.4
Nitrogen	1.25	17.4	−195.8	−146.8	3.4
Oxygen	1.43	20.2	−182.9	−118.4	5.1

* At atmospheric pressure

** Sublimation point

Table 8.9

Electric resistivity of some metals and resistor materials at 20 °C
($\Omega\ mm^2\ m^{-1}$)

Aluminium	0.03	Bronze	0.01
Iron	0.1–0.15	Constantan	0.49
Lead	0.21	Kanthal	1.1–1.45
Platinum	0.10	Manganine	0.43
Silver	0.02	Nickel-silver	0.3–0.36
Tungsten	0.06		

Table 8.10

Electric conductivity of NaCl solution at 20 °C

Concentration (c) mol/litre	Specific conductivity ($\varkappa$) $\Omega^{-1}\ m^{-1}$	Equivalent conductivity $\left(\Lambda = \frac{\varkappa}{c}\right)$
5	22.2	4.4
3	17.8	5.9
2	13.5	6.8
1	7.7	7.8
0.5	4.2	8.5
0.1	1.0	9.7
0.05	0.5	9.6
0.01	0.1	10.0
0.005	0.05	10.4
0.001	0.01	10.7

Table 8.11

Refractive indices of some materials for light of wavelength 589 nm (Na D line) at 20 °C

Ethanol	1.360
Glass	1.517–1.890
Rock salt	1.544
Silica glass	1.459
Water	1.333

Table 8.12

Some data on biological substances

Density ($kg\ dm^{-3}$)		Specific electric resistivity at 30 MHz frequency ($\Omega^{-1}\ m^{-1}$)	
Bone	1.7–2.0	Blood	≈1.1
Cartilage (average)	≈1.1	Muscle	≈0.8
Fatty tissue	0.92–0.94	Spleen	≈0.6
Blood cells	≈1.1	Liver	≈0.5
Plasma	≈1.03	Brain	≈0.45
Blood (average)	≈1.06	Fatty tissue	≈0.05
Urine	1.001–1.035		
Viscosity relative to water (at 20 °C)			
Plasma	1.8–2.0		
Blood (average)	4.2–6		
Cytoplasm	≈45		
Endolymph	≈1.8		
Specific heat ($kJ\ kg^{-1}\ K^{-1}$)		Dielectric constant at 30 MHz frequency	
Blood	≈3.9	Blood	≈140
Compact bone	1.3–1.7	Muscle	≈110
Fatty tissue	≈3	Spleen	≈200
Body tissue (average)	≈3.5	Liver	≈140
		Brain	≈160
		Fatty tissue	≈ 12

Table 8.13

Characteristic data on some important radionuclides

Chemical element and its atomic number		Isotope symbol	Physical half-life	Type of decay	Maximum particle energy (MeV)	γ-energy (MeV)
Hydrogen	1	^{3}H	12.33 years	β^-	0.0186	—
Carbon	6	^{11}C	20.4 min	β^+	0.96	—
		^{14}C	5760 years	β^-	0.155	—
Nitrogen	7	^{13}N	10 min	β^+	1.19	—
Oxygen	8	^{15}O	2 min	β^+	1.73	—
Fluorine	9	^{18}F	109.8 min	β^+	0.633	—
Sodium	11	^{24}Na	15.02 hours	β^-, γ	1.392	2.754 1.369
Phosphorus	15	^{32}P	14.28 days	β^-	1.710	—
Sulfur	16	^{35}S	87.2 days	β^-	0.167	—
Potassium	19	^{40}K	$1.28 \cdot 10^9$ years	β^-, K (10%)	1.31	1.46 after K
		^{42}K	12.36 hours	β^-, γ	3.52 (75%) 1.99 (25%)	1.525
Calcium	20	^{45}Ca	163 days	β^-	0.257	—
Chromium	24	^{51}Cr	27.7 days	K, e^-, γ	0.315 (e^-)	0.320
Iron	26	^{52}Fe	8.2 hours	β^+, γ	0.8	0.5 1.30
		^{59}Fe	44.6 days	β^-, γ	1.566	1.10
Cobalt	27	^{60}Co	5.272 years	β^-, γ	0.318	1.33 1.17
Copper	29	^{64}Cu	12.74 hours	β^- (39%) β^+ (19%) K (42%) γ (1%)	β^-: 0.575 β^+: 0.656	 1.34
Krypton	36	^{85}Kr	10.73 years	β^-, γ	0.687	0.514
Rubidium	37	^{81}Rb	4.7 hours	β^+, γ	0.99	1.93 0.95
		^{86}Rb	18.65 days	β^-, γ	1.78	1.078
Strontium	38	^{90}Sr	29 years	β^-	0.546	—
Yttrium	39	^{90}Y	64 hours	β^-, γ (0.4%)	2.29	1.761
Technetium	43	$^{99}Tc^m$	6.02 hours	γ	—	0.140

Table 8.13 (continued)

Chemical element and its atomic number		Isotope symbol	Physical half-life	Type of decay	Maximum particle energy (MeV)	γ-energy (MeV)
Indium	49	$^{113}In^{m}$	1.658 hours	γ	—	0.391
Iodine	53	^{123}I	13.3 hours	γ	—	0.16
		^{125}I	59.7 days	K, γ	—	0.0355
		^{131}I	8.04 days	β^-, γ	0.606	0.364
					0.25	0.080
					0.81	0.723
Xenon	54	^{133}Xe	5.29 days	β^-, γ	0.346	0.081
Caesium	55	^{137}Cs	30.1 years	β^-, γ	0.512(92.6%)	0.661
					1.173 (7.4%)	
Gold	79	^{198}Au	2.695 days	β^-, γ	0.961	0.411
Mercury	80	^{203}Hg	46.6 days	β^-, γ	0.212	0.279
Radon	86	^{222}Rn	3.824 days	α	5.489	—
Radium	88	^{226}Ra	1600 years	α, γ (6%)	4.784	0.186
						0.260
					4.598	0.609
Uranium	92	^{238}U	$4.47 \cdot 10^{9}$ years	α, γ	4.2	0.048

Table 8.14

Fundamental physical constants

Velocity of light in vacuum	$c = 2.998 \cdot 10^{8}$ m s^{-1}
Universal gas constant	$R = 8.314$ J mol^{-1} K^{-1}
Avogadro constant	$N_A = 6.02 \cdot 10^{23}$ mol^{-1}
Boltzmann constant	$k = 1.38 \cdot 10^{-23}$ J K^{-1}
Electron rest mass	$m_e = 9.11 \cdot 10^{-31}$ kg
Proton rest mass	$m_p = 1.67 \cdot 10^{-27}$ kg
Elementary charge	$e = 1.6 \cdot 10^{-19}$ C
Planck constant	$h = 6.62 \cdot 10^{-34}$ J s
Faraday constant	$F = 96{,}485$ C mol^{-1}

SUBJECT INDEX

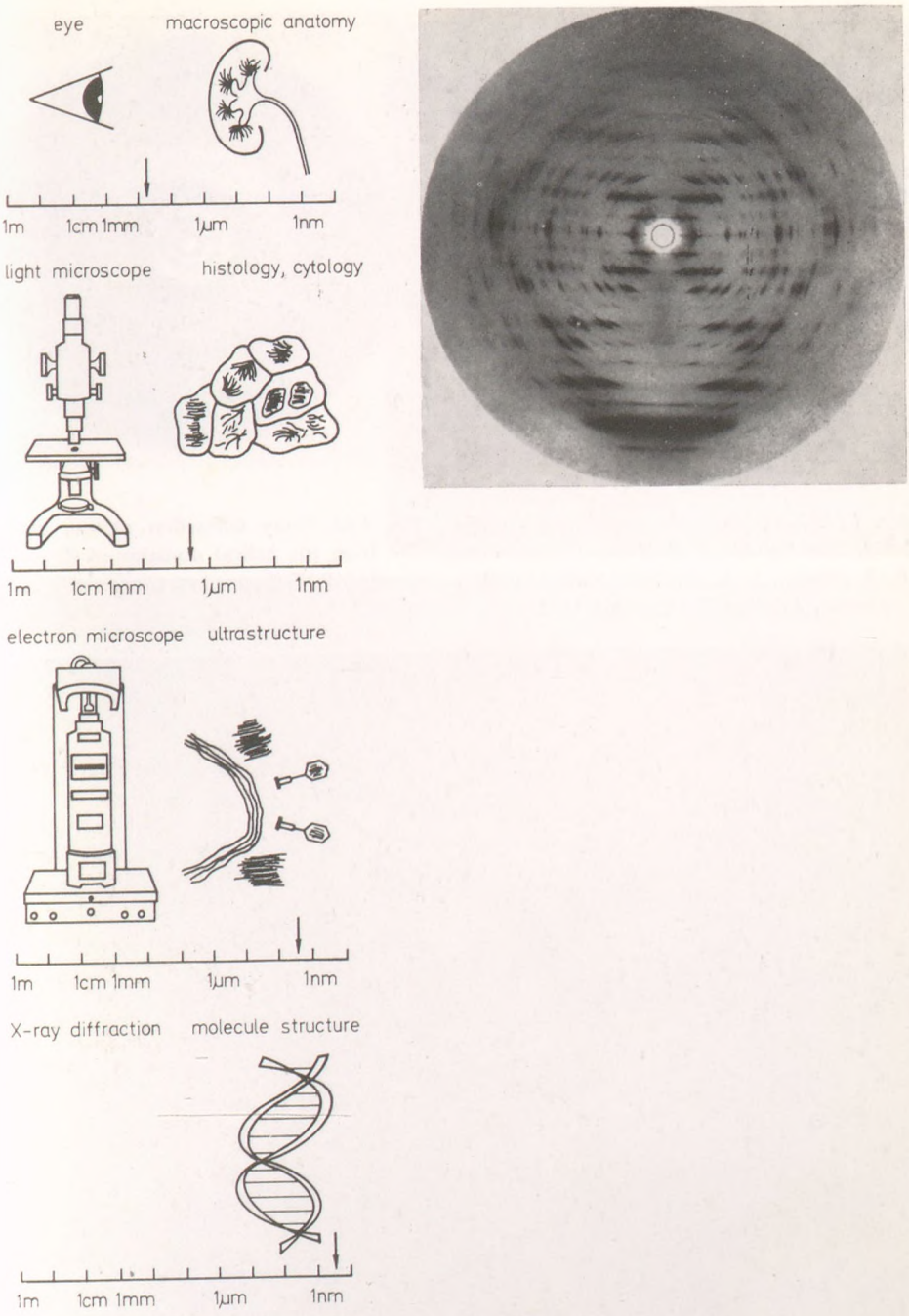


Fig. 1.1. Some of the more important stages in the development of structure analysis
The arrows on the scales indicate the smallest dimensions of details dctectable with the individual methods. The X-ray diffraction photograph is shown in the upper right corner

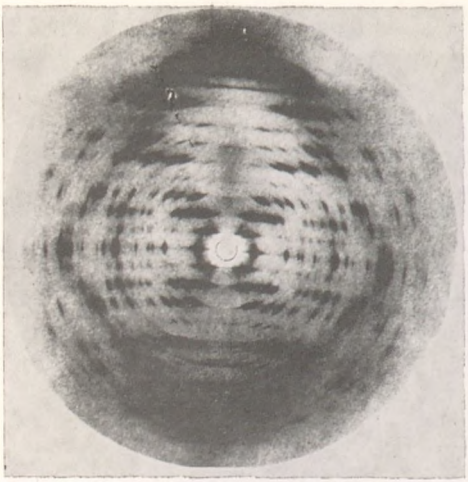


Fig. 1.32. X-ray diffraction pattern of a single DNA fibre (by *M. H. F. Wilkins*; taken from *R. B. Setlow, E. C. Pollard*: Molecular Biophysics, Addison-Wesley Publ. Co., 1962)

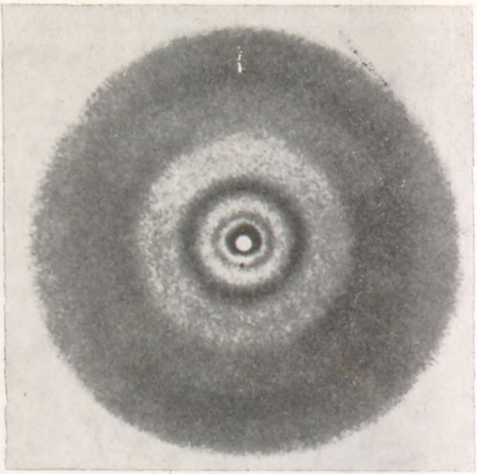


Fig. 1.33. X-ray diffraction pattern obtained from the helical domains of *E. coli* tyrosine-tRNS (by kind permission of *W. Fuller*)

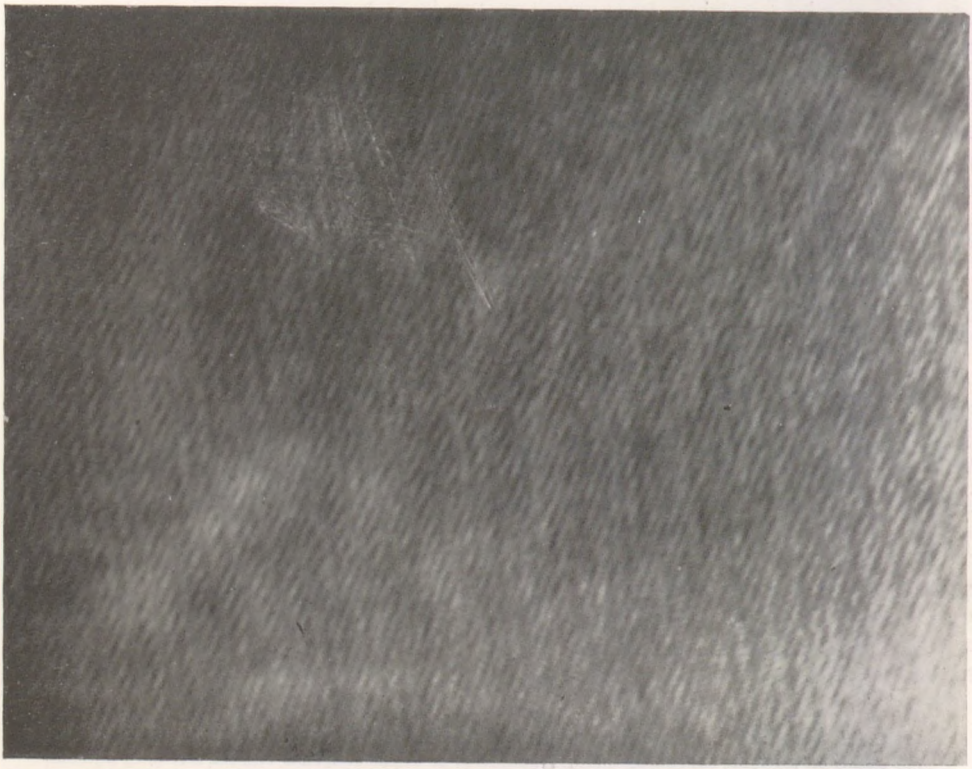
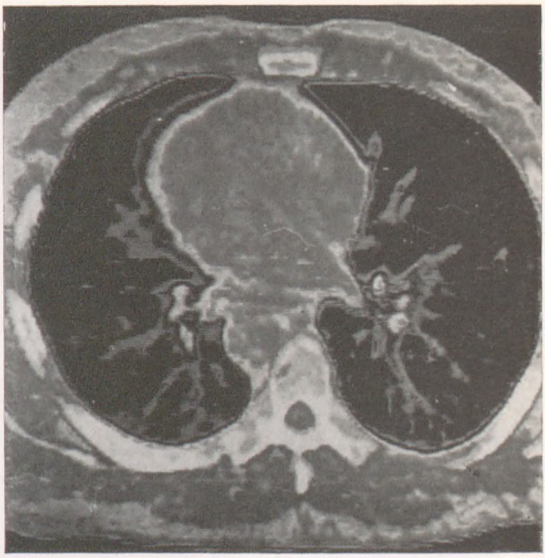


Fig. 2.13. Hologram of a standing woman

Fig. 2.31. Densitogram of a section through the chest
The vertebra, heart, great arteries, lung and bronchi are clearly seen



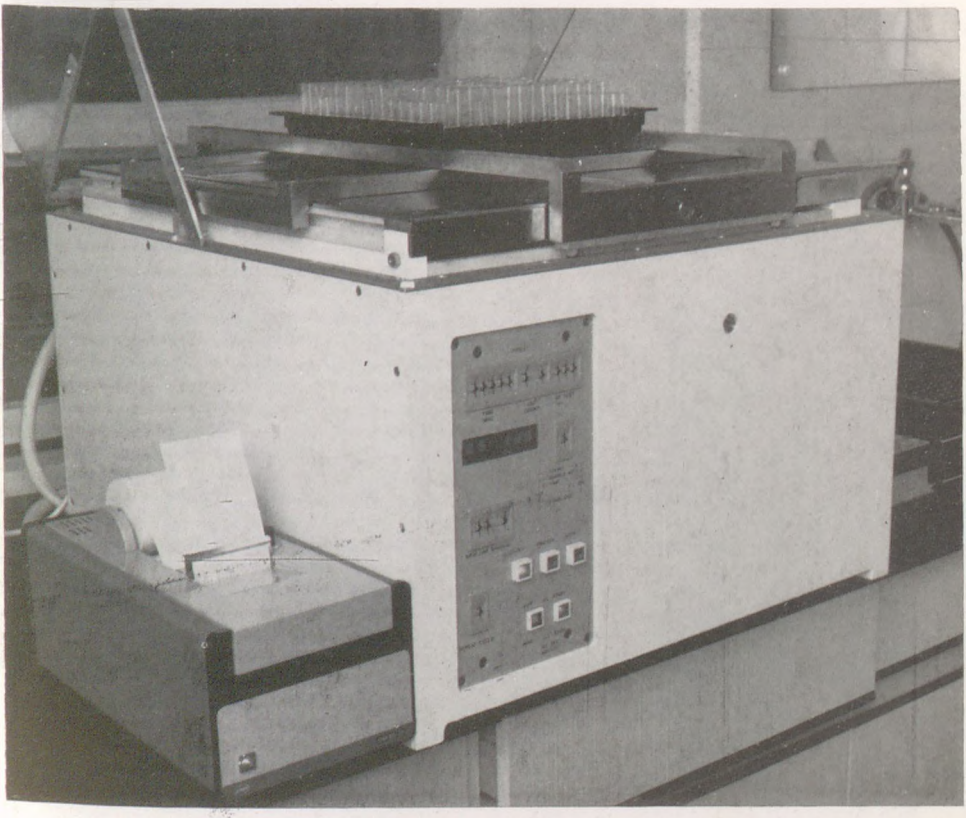


Fig. 2.44. Counter with digital display and lead shielding for the preparation to be measured (GAMMA Művek, Hungary). The scintillation detector is in the upper part of the lead shield

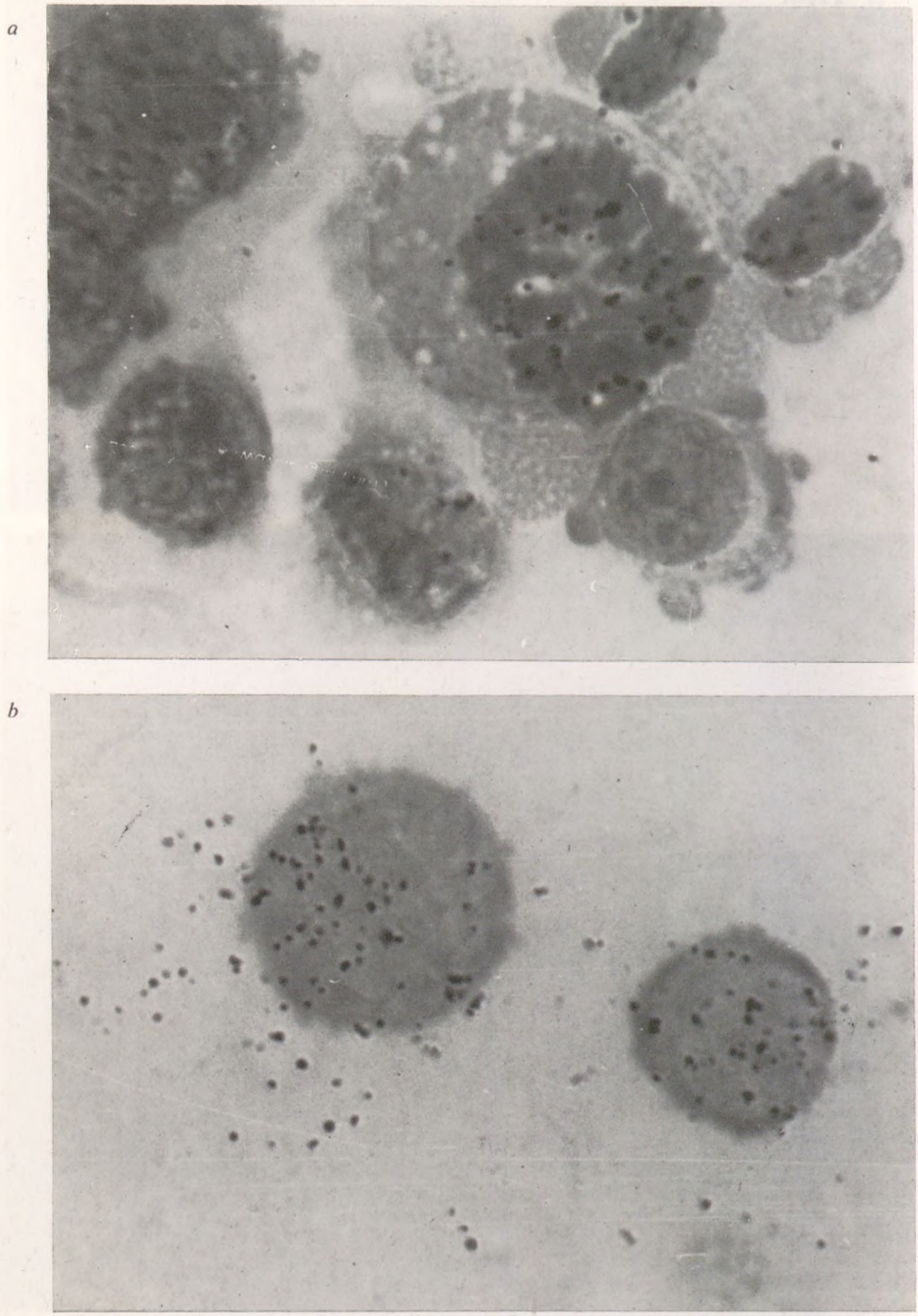


Fig. 2.45. Microautoradiogram of mouse-ascites lymphoma (NK) cells labelled with ^{3}H-thymidine (*a*) and ^{14}C-thymidine (*b*) (photographs by *L. Varga*)

a: Ilford G5 emulsion, Giemsa staining, magnification 1500×, *b:* Ilford G5 emulsion, methyl green-pyronine staining, magnification 1500×

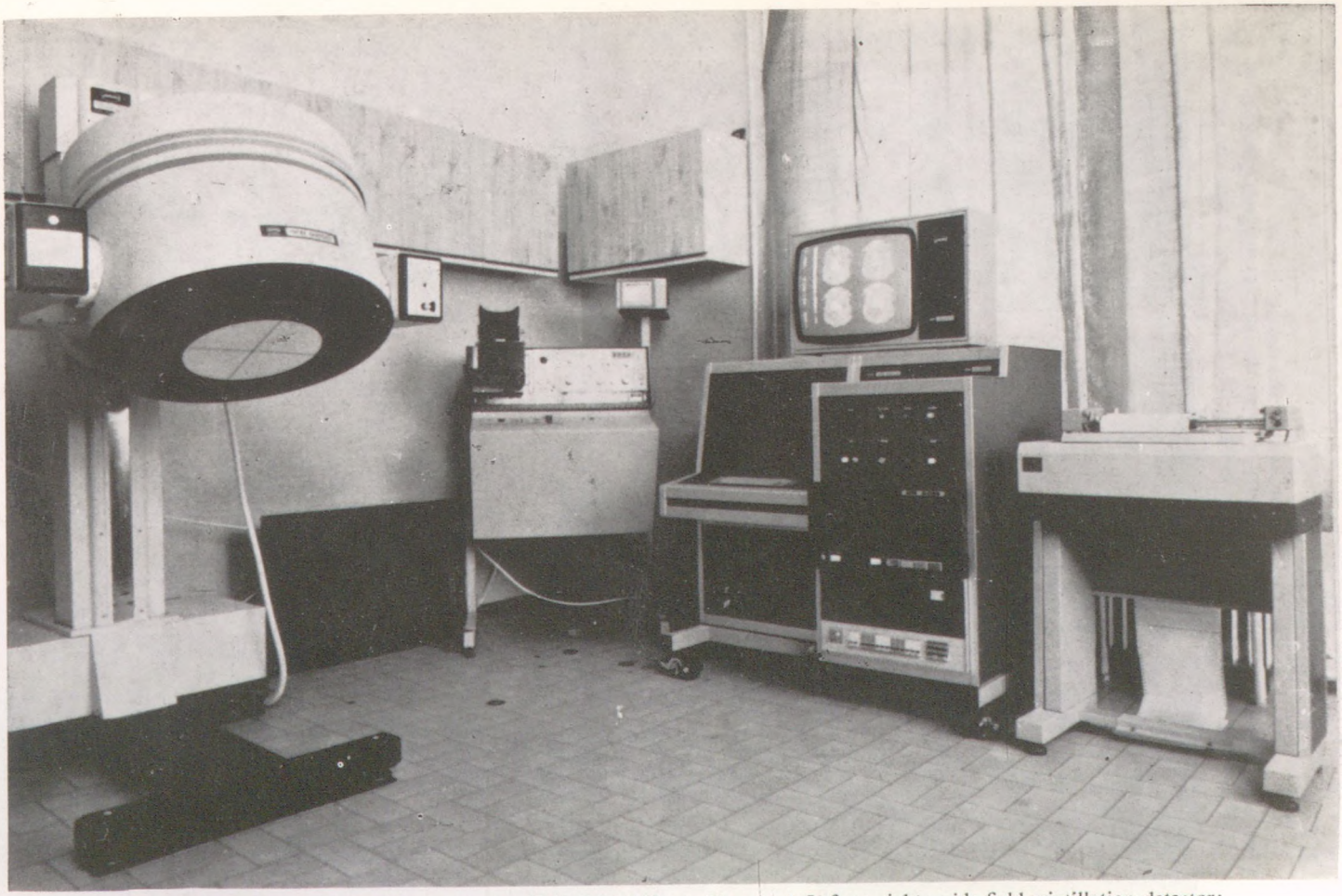


Fig. 2.55. Gamma-camera with accessories (GAMMA Művek, Hungary). Left to right: wide-field scintillation detector; electronic signal processor, information processing computer and display unit; graphic printer

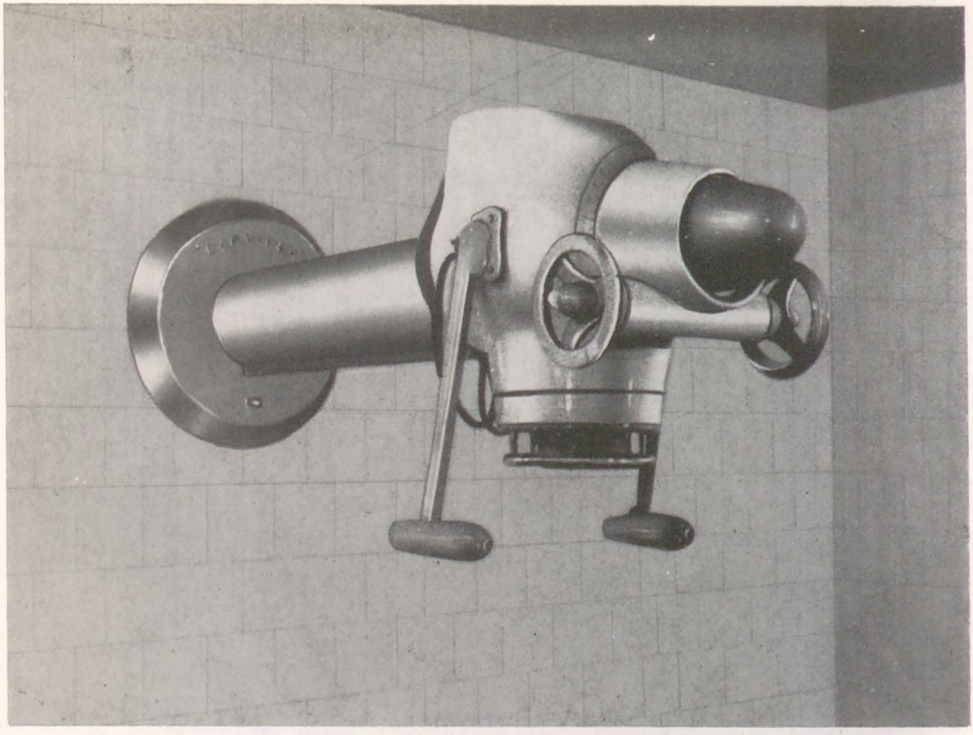


Fig. 2.61. The irradiating head of the Gravicert (Medicor) cobalt gun revolving around a horizontal axis

After each irradiation the cobalt charge slides back from the irradiating head through the supporting arm to the wall storage. The two down-reaching side-arms permit simple optical determination of the distance of the body to be irradiated; the size of the radiation field is defined by the exchangeable lead diaphragms on the bottom of the irradiating head. Maximum charge: 110 TBq (≈3000 curie) $^{60}_{27}Co$

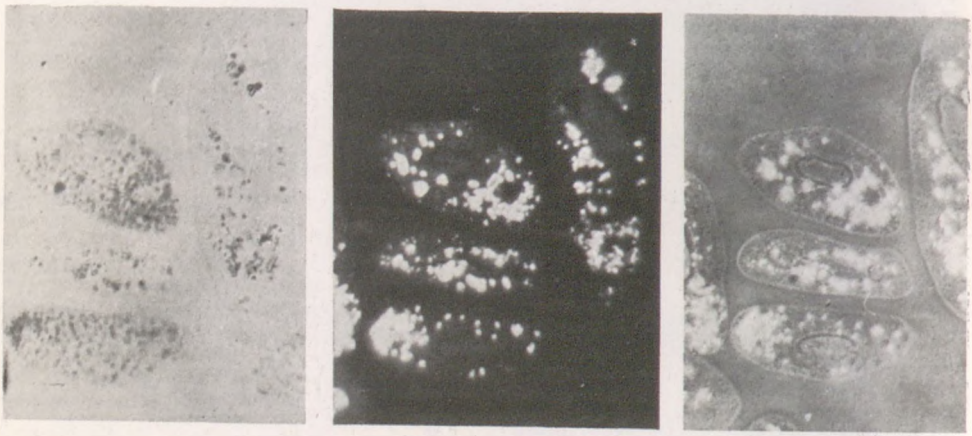


Fig. 3.5. Microscopic images of protozoa

a: simple light field image; *b:* dark field image; *c:* phase contrast image (from *M. J. Pelczar, R. D. Ried,* Microbiology. McGraw-Hill, New York 1965)

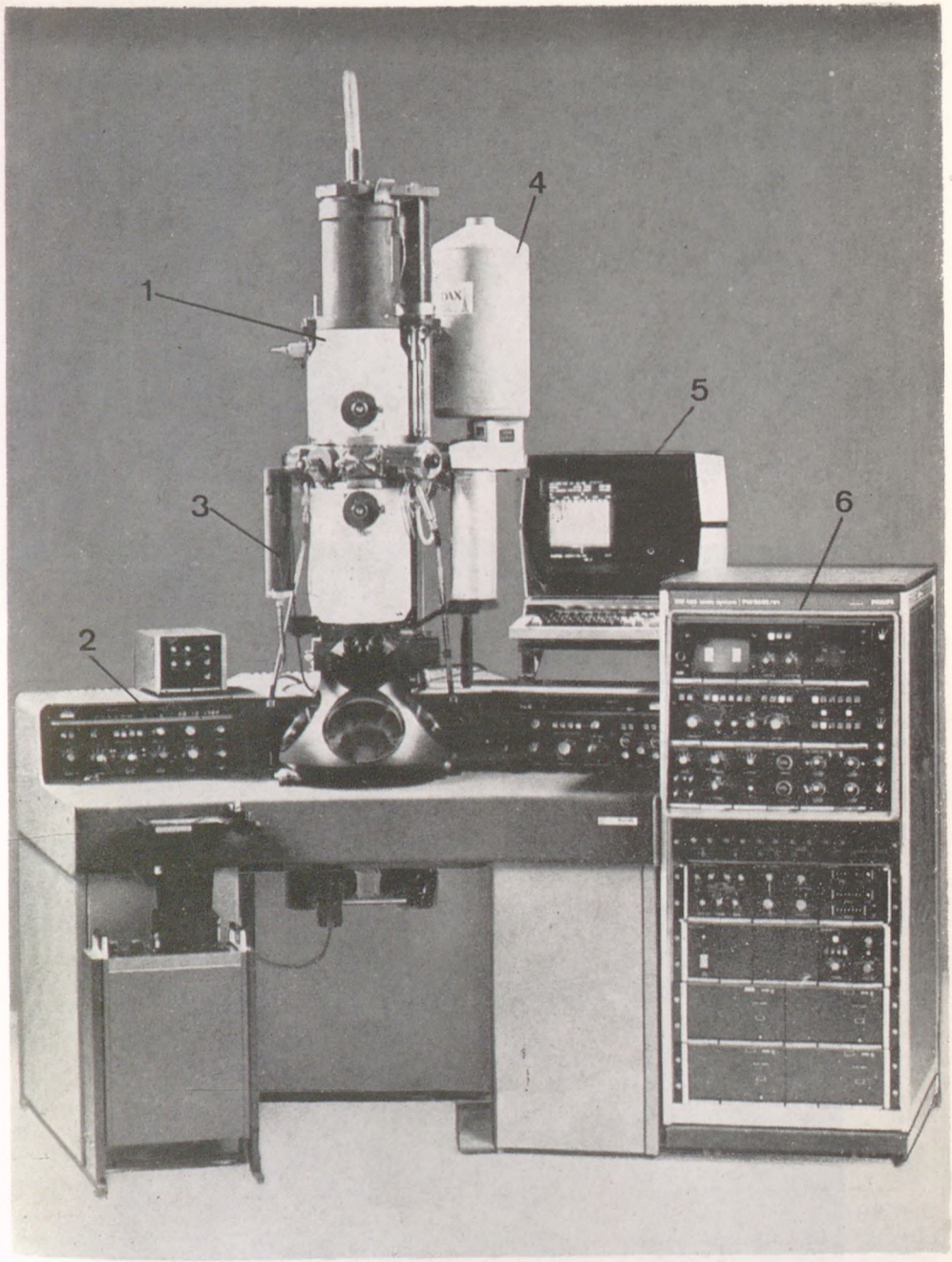


Fig. 3.10. Electron microscope EM420 (Philips)

1: transmission electron microscope; *2:* operating console with service accessories; *3:* secondary electron detector; *4:* X-ray detector; *5:* X-ray microanalyser with display; *6:* electronics for the scanning mode

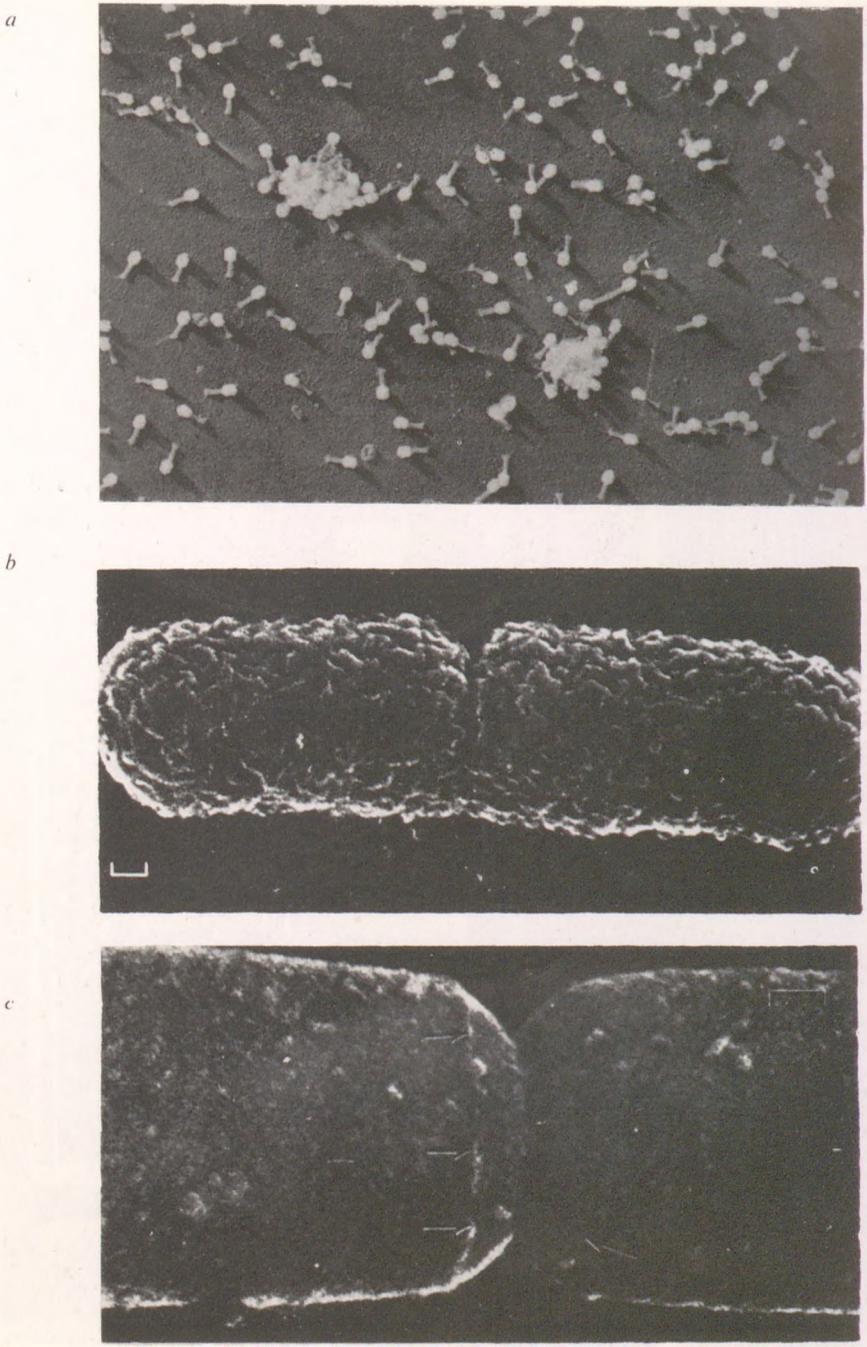


Fig. 3.11. Electron micrograms

a: electron micrograph of T2 phages, obtained with a traditional electron microscope but using special contrast and plasticity-improving technique (magnification: 25,000×; *R. M. Herriott, J. L. Barlow,* J. Gen. Physiol., *36*, 17, 1952); *b–c:* scanning electron micrograms of dividing bacteria (*E. coli* and *B. subtilis*). The arrows indicate the ridges separating the old and new surface areas produced in the division (*K. Amako, A. Umeda,* J. Gen. Microbiol., *98*, 297, 1977)

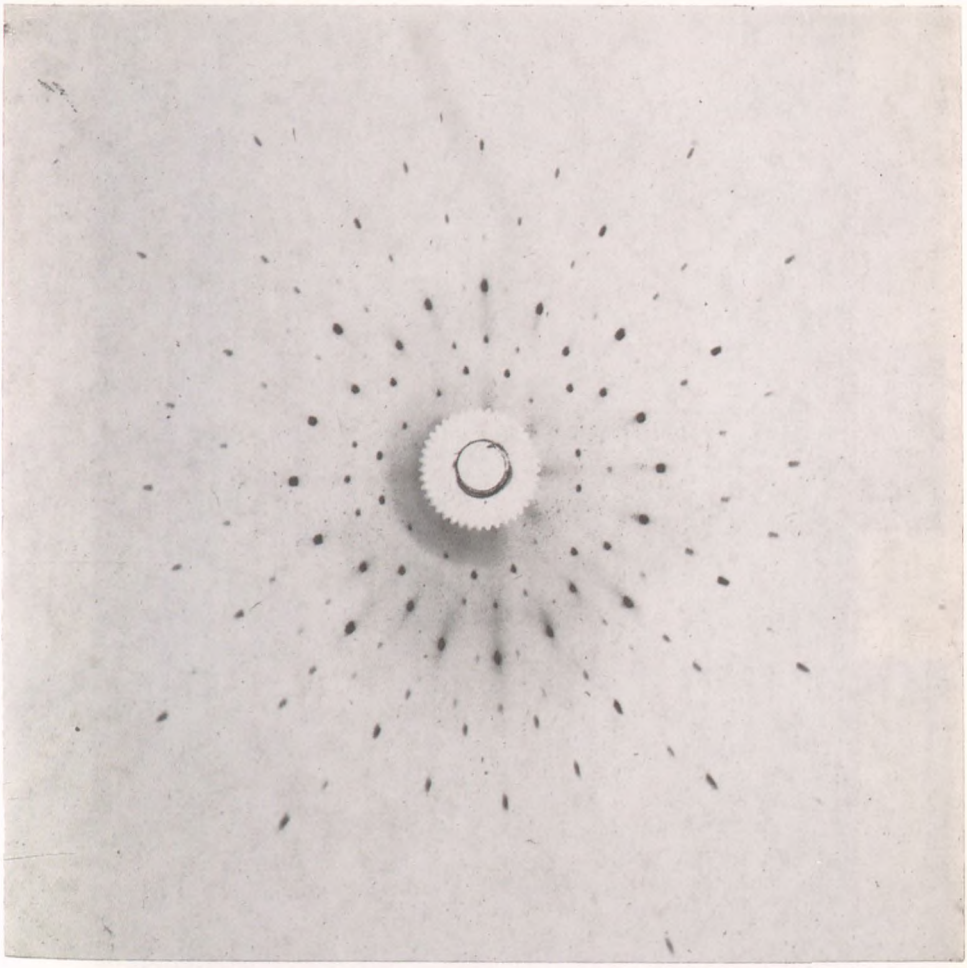


Fig. 3.23. Laue diagram of a NaCl crystal (photograph by courtesy of *L. Varga*)

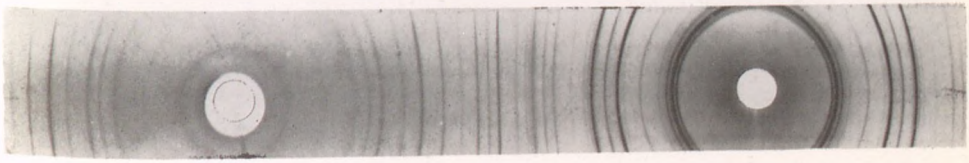


Fig. 3.25. Debye–Scherrer diagram of crystalline ZnS powder (photograph by courtesy of *M. Jahnke*)

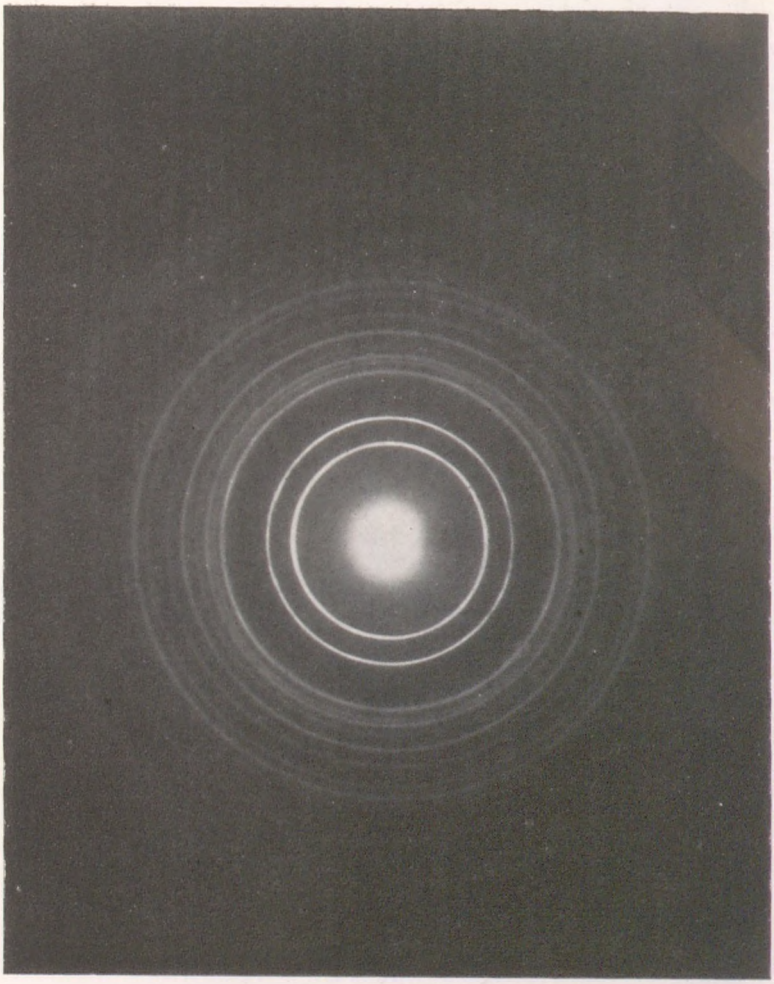


Fig. 3.26. Diffraction of an electron beam on a thin gold foil (photograph by courtesy of *A. Barna*)

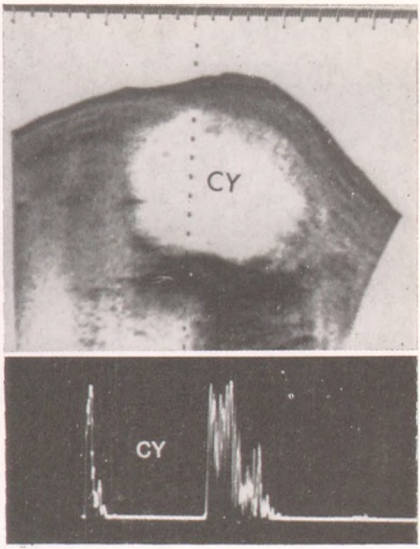


Fig. 5.32. Echogram of a cyst (CY)
Above: a two-dimensional gray-scale *B* image; below: an *A* image. The latter relates to the dashed line on the *B* image (Department of Radiology, Semmelweis University of Medicine)

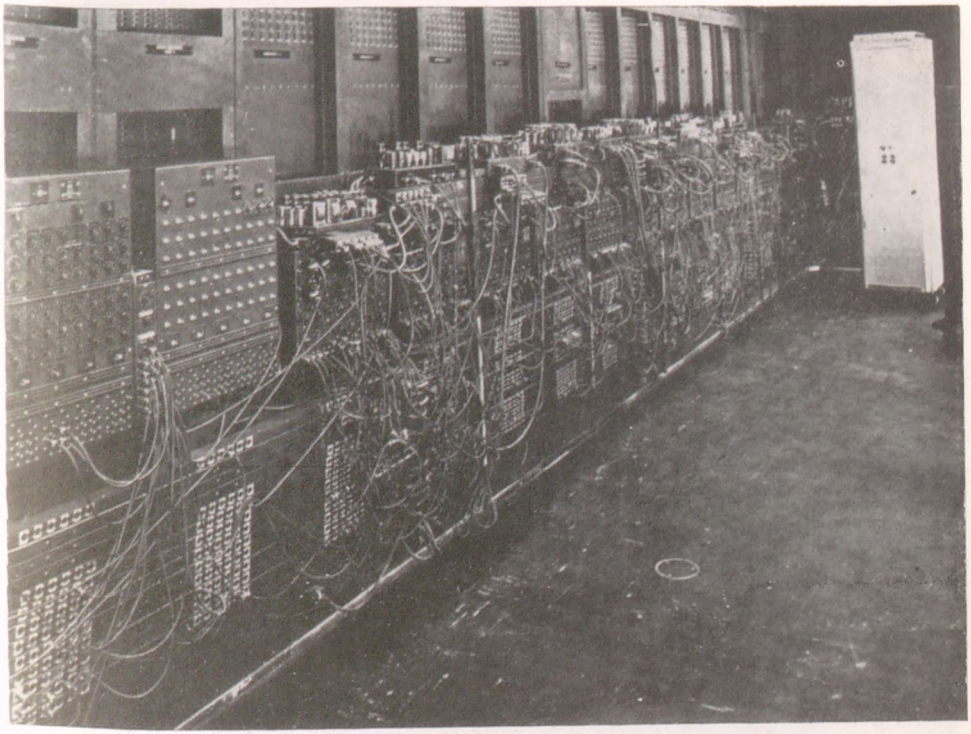


Fig. 7.12. ENIAC, first large-scale electronic digital computer

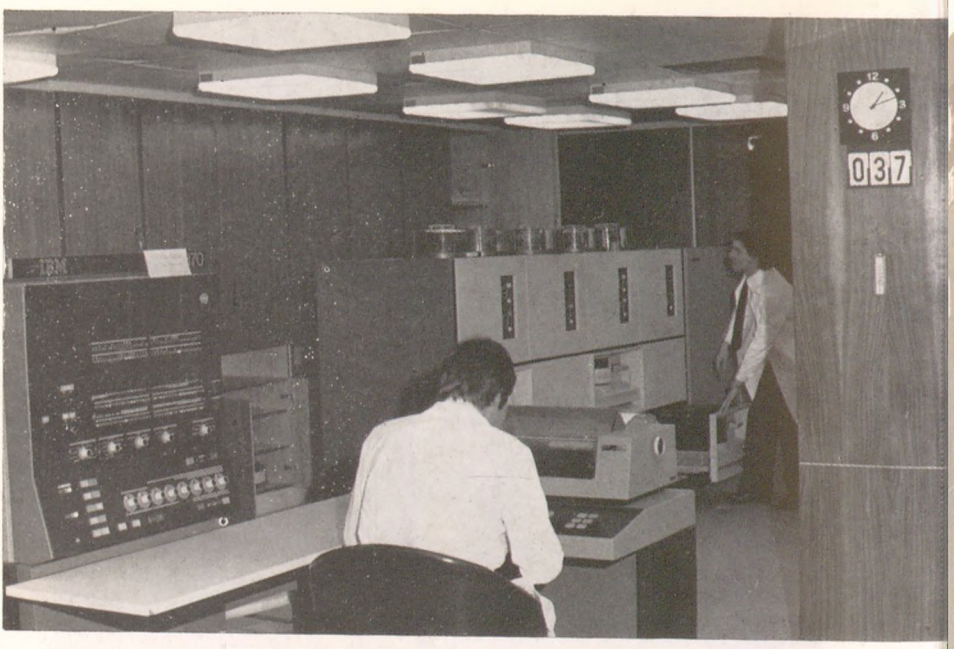


Fig. 7.13. The machine room of an IBM computer
On the *left* the central processor unit, on the *right* the external storage units. The typewriter-like device serves for information input

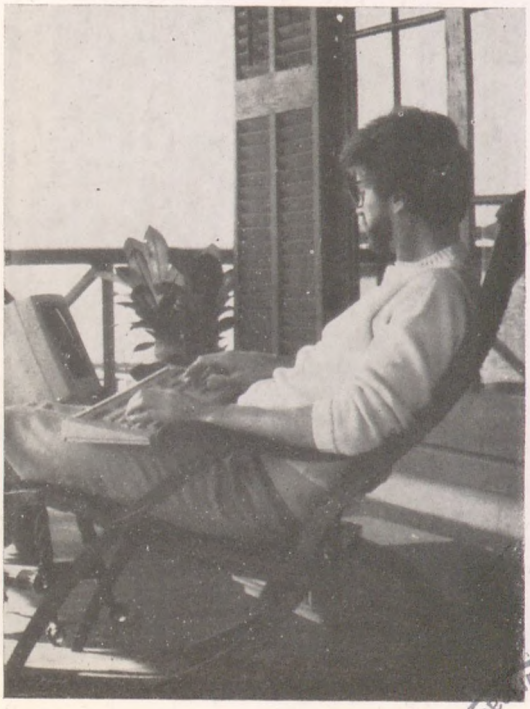


Fig. 7.14. Personal computer with its display

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WITH MEDICAL ORIENTATION

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