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CONTENT

8. THE ROLE OF GENETIC FACTORS IN THE DEVELOPMENT OF DISEASES

8.1. INTRODUCTION

The external causes of diseases are manifold and cover a wide range of agents - from mechanical injuries to protozoa. In many cases, however, diseases manifest without apparent external damage (Tab. 8.1).

Tab. 8.1. Diseases without apparent external cause

hypertension
diabetes mellitus
coronary heart disease
madness
hemophilia
inborn errors of metabolism
endocrine diseases
dysfunction of the immune system
malignancies

Despite tremendous complexity of the human body at last only one type of truly internal cause exists - errors in the genetic code. Although alredy in the ancient times everybody knew that children usually resemble their parents, the role of inheritance in the development of human diseases is a relatively new development (Tab. 8.2).

Tab. 8.2. The cornerstones of medical genetics

ANCIENT TIMES
children resemble their parents,
breeding of domestic animals
INHERINTANCE OF DISEASES
1750 Maupertuis - polydactyly
1820 Nasse - hemophilia
1865 Mendel - basic laws of genetics
1876 Galton - twin method, polygenic inheritance
1908 Garrod - inborn errors of metabolism
1953 Watson and Crick - DNA structure
1970 genetic engineering
1990 Human Genome Project (HUGO)

The internal set or library of information is the basic and necessary prerequisite of every phenomena interconnected with life (reproduction, development, metabolism, adaptation, regulation, compensation). The material basis and the code of heredity is known (chromosomes, genes, DNA and RNAs, nucleotide triplets) and more and more information is gained on its function (transcription, translation, proof reading, repair systems, etc.) However, one should realize, that this huge library of information is a nonliving and meaningless stuff without its environment. It can realize itself only with interactions within the cell, with other cells, tissues and the outer world. The chromosome proteins (histones amd chromatin) are not merely envelopes of the DNA double helix and the ribosomes are not only supporting and mechanical assembly particles for the proteins but they exert very strong regulatory functions.

The human genome contains about 100 000 genes which code information for an equivalent amount of proteins from which then act as intra- or extracellular enzymes, receptors, transport and signal systems, clotting factors, structural scaffold of the body (e.g. collagen) and regulatory proteins involved in metabolic regulations or development.

In addition to structural genes large amount (more than 95%) of DNA consist of noncoding sequences whose function is as yet not clear, but the regulatory genes accounting for proper expression of structural genes are surely among them. Other noncoding sequences are probably responsible for chromosome integrity.

More than 4000 genes have already been identified, and barely a week goes by without the announcement of a new addi-

tion to the repertoire. The target of the Human Genome Project developed in 1990 by NIH (National Institues of Health) and DOE (Department of Energy) in the U.S.A is to draw the detailed map the human genome by the year 2005. The progress toward achieving this goal after the first 5 years appears to be on schedule or, in some instances ahead of schedule due to substantial improvement in technologies (gene mapping, sequencing and identification methods) and better information processing, storage and transfer.

It is obvious that errors occuring anywhere in the whole system can lead to disease but the way from a genetic fault to a certain disease is very complex and not a straightforward one.

Theoretically every error in every structural and regulatory gene can lead to disease, but the actual number of "genetic" diseases is much lower. In 1986 McKusick listed phenotypic variations or diseases of about 2000 established genetic loci. The discrepancy between the hypothetical astronomic number and the actually known genetic diseases can be explained as follows:

* **Genetic variations account for physiological differences between people** (height, hair and skin color*, blood groups, etc.). More than 25 % of all human genes are polymorphic and the process of genetic recombination between different alleles is the most important prerequisite for the development and future survival of the mankind.

*According to scientific evidence the racial genetic differences are only "skin deep" and the diversity among individuals belonging to a racial group is much greater. If genes for surface traits (skin color, stature) are discounted, the human "races" are almost identical. The concept of "race" is therefore meaningless at the genetic level. There is no scientific basis for theories touting genetic superiority of any one population over another

* **Some genetic variations are not compatible with life and therefore do not cause diseases.**

* **The interaction between genes and environment is very complex.** Genetic variations can be anticipated in the background of a number of "nongenetic" common diseases listed in Tab. 8.1.

* **The same phenotype can be caused by different mistakes in a given gene** as for example in the thalassemias or in the gene responsible for cystic fibrosis (350 mutations described as far for this disease!).

TYPES OF DISEASES CAUSED BY GENETIC DISORDERS

There are three principal types of diseases caused by disorders of the genetic code:

1. Single gene mistakes causing diseases that obey the Mendelian rules of genetics.

2. Genetic variations which do not cause disease in itself but rise the probabilty of their manifestation under certain circumstances.

3. Chromosomal aberrations.

The frontiers between these groups are not sharp. For example high cholesterol level can be a consequence of a single genetic error in LDL-receptor gene (group 1) or may arise as a combination of some variant genes of lipid metabolism and unhealthy life style (group 2). The genes cannot work properly neither in the case of chromosome translocation (which is visible in electron microscope - group 3) nor after some single point mutations (detectable only by methods of molecular biology - group 1).

In addition to these three main groups of hereditary diseases recently two further types of hereditary disorders we-

re described:

4. Recent development in understanding the function of noncoding regions of the genome enables to establish an up to date short and uncertain list of diseases arising as consequences of errors in the noncoding region of the genome. This list will be probably much longer and less hypothetical in the near future. (Tab. 8.3).

5. The nucleus is not the only place where DNA can be found. Mitochondria contain a small set of genes (for some enzymes of the terminal oxidation). The basic features of these genes differ from those in the nucleus and therefore the diseases caused by mistakes in mitochondrial genes do not obey the rules of Mendelian genetics.

Tab. 8.3. Noncoding regions of the genome and disease

THE BASIC AXIOMS * 97 % of the genome is noncoding but probably not "junk" * The chromosomes are complex information organelles * 1 strucural gene - at least 5 regulatory gene * Sophisticated maintenance and control systems
KNOWN EXAMPLES Thalassemia - failure of regulatory genes for Hb gene expression. Fanconi's anemia - failure of the replication control ? Fragile X-chromosome - abnormal DNA methylation ?
LIST OF KNOWN NONCODING SEQUENCES AND THEIR ROLE IN PHYSIOLOGY AND PATHOLOGY * introns - noncoding sequences within genes role in ribosome assembly and/or gene expression ? * satellites - short sequences repeated 100-1000 times at the end or center of chromosomes role in chromosome stability * mini- and microsatellites defects associated with cancer ? * SINE-s and LINE-s Short (≈ 300 base pairs) and long (≈ 7000 base pairs) intervening sequences repeated at many sites of the genome. At the wrong site can cause disease e.g. neurofibromatosis * 3'utr (untranslated region) controls the rate of mRNA degradation faulty 3'utr - myotonic dystrophy ?

Sometimes the difference between hereditary and congenital diseases is not properly understood. Most, but not all monogenic hereditary diseases manifest in early life. (They are both hereditary and congenital.) In some cases, however, the hereditary disease is not apparent at birth although the error in the genome is present. These diseases are hereditary but not congenital (e.g. accelerated atherosclerosis and its sequale in heterozygotes for faulty LDL-receptor gene).

Chromosomal aberrations, on the other side, arise mostly during the first divisions of the fertilized egg. They represent errors of the genetic information, are congenital but (with some exceptions) not hereditary.

Some further congenital pathologic conditions do not fit the frame of the diseases caused by genetic factors. External agents can affect the development of the fetus during intrauterine life and lead to serious birth defects and diseases without changes in the genotype (Tab. 8.4).

Tab. 8.4. Congential malformations due to external factors

PHYSICAL FACTORS
Injuries
Ionizing radiation
CHEMICAL FACTORS
Alcohol
Smoking
Drugs - thalodimide (limb malformations)
Dioxin, polychlorinated biphenyls
Methyl mercury
BIOLOGICAL FACTORS
Viral infections - rubeola (heart malformations)
Diabetes mellitus - hyperglycemia
during organogenesis - malformations
2nd and 3rd trimester - big newborns

8.2. MONOGENIC DISEASES

Disorders caused by single gene mutations can be divided according to the type of inheritance and the affected chromosomes to:

* **Autosomal dominant traits**
* **Autosomal recessive diseases**
* **Sex-chromosome linked disorders**

Dominant traits are expressed in heterozygotes, recessive only in homozygotes but the meaning of these terms depends also on the level of observation. Sickle cell anemia causes serious clinical symptoms only in homozygotes (recessive trait) but in heterozygotes analysis of the blood reveals the presence of both hemoglobin A and S. From this biochemical point of view the presence of Hb S is a codominantly inherited trait.

There are about 2000 known monogenic diseases, but on the other side there are relatively rare (Tab. 8.5). In some cases the possibility of a new mutation cannot be excluded.

Tab. 8.5. Examples of monogenic diseases

OVERALL FREQUENCY ≈ 10/1000 live births (PROBABILITY OF NEW MUTATIONS ≈ 1/100 000)		
≈ 7	dominant	
	Familial hypercholesterolemia (faulty LDL-receptor gene)	1/ 500
	Polycystic kidney	1/ 1250
	Marfan's sy. (collagen)	1/ 20000
≈ 2.5	recessive	
	Sickle cell (Hb S)	1/ 625 in US blacks
	Cystic fibrosis	1/ 2000 in Caucasians
	Tay-Sach (lipid met.)	1/ 3000 in Jews
	Phenylketonuria	1/25000
≈ 0.4	X-linked	
	Hemophilia A	1/10000
	Duchenne m. dystrophy	1/7000
	G6PD deficency	
	and	
	Color blindness	8 % of Caucasian males

AUTOSOMAL DOMINANT TRAITS

These disorders are fully manifest if only one of the chromosomes is affected. According to Mendelian rules of genetics:

* **Each affected individual has an affected parent;**
* **about half of the offspring is affected;**
* **males and females are affected in equal proportions.**

In addition most autosomal disorders show marked variability in severity and delayed age of onset. This enables the transmission of the faulty gene to the next generations. (Transmission of a lethal gene or a gene causing infertility as dominant trait is impossible.) When a gene of a dominant trait occur in homozygous form, the effect may be very severe disease (very high cholesterol in homozygotes for faulty LDL-receptor gene) and early death.

AUTOSOMAL RECESSIVE DISORDERS

Clinically apparent disease occur only in homozygous state, when both alleles are faulty. According to Mendelian rules of genetics:

* **The parents are clinically healthy;**
* **from the offspring of two healthy heterozygotes: one out of four is affected with the disease every second is healthy heterozygous carrier of the trait;**
* **males and females are affected in equal proportions.**

Marriage between relatives (consanguinity) considerably increase the probability of the occurence of such diseases. One should keep in mind that everybody has some (approximately 3 - 8) faulty genes in his genome! Refined biochemical or genetical methods enable identify these hidden abnormalities and make genetic counseling possible.

The recessively inherited diseases are mostly identical with the enzyme deficiencies (or inborn errors of metabolism), hemoglobinopathies and deficiencies of specific blood proteins. They are usually rare but severe diseases and the affected persons without special medical treatment usually die in young age or cannot have children. This, however, does not affect the transmission of the faulty gene to the next generations by healthy heterozygotes (carriers). In some cases environmental factors can affect the occurence of recessive disorders. Heterozygotes for sickle cell trait are relatively resistant to malaria and due to this external selective force this disease (similarly as the X-linked G6PD deficiency) is relatively frequent in parts of the word where malaria was endemic in the past.

SEX CHROMOSOME LINKED INHERITANCE

The most common form of the sex chromosome linked inheritance is the X-linked recessive mode.

* The disorder is fully expressed in the affected males because they have only one X-chromosome;

* the affected males transmit the faulty gene to all their daughters (their sons receive the Y-chromosome);

* heterozygous females are usually clinically healthy but they are carriers of the trait;

* carrier heterozygous females transmit the trait to 50% of their sons.

In some cases heterozygous females can express mild symptoms of the disease. This is attributed to the fact, that in their cells only one of the X-chromosomes is active, the second is inactivated in random way during early intrauterine life (Lyon hypothesis). The severity of symptoms therefore

correlate with the percentage of the inactivated normal X-chromosome.

In addition to this frequent type of inheritance, (color blindness, hemophilia A and B, G6PD deficiency) X-linked dominant traits are also described. In this case the trait is manifest both in heterozygous females and hemizygous males. Examples include vitamin D-resistant rickets, pseudohypoparathyroidism and one of the blood group antigens. The only genes currently known to be located on the Y chromosome **(holandric genes)** are those that determine male sex and an antigen involved in graft rejection after organ transplantation. These genes are transmitted from fathers to their sons.

Not every sex-linked phenotypic trait is coded on the sex chromosomes. Baldness, for example, is an autosomal dominant trait but its manifestation is influenced by sex through production of testosterone.

MITOCHONDRIAL INHERITANCE

Human mitochondria contain circular chromosomes that code for certain RNAs and 13 proteins involved in terminal oxidation. The organization of mitochondrial DNA resembles those of bacteria (wall-to-wall genes without introns) and its mutation rate is much higher than that of nuclear DNA. Although spermatozoa contain mitochondria, the transmission of mitochondrial genes is exclusively maternal.

Defects in mitochondrial genes cause certain myopathies (e.g. ragged-red fiber myopathy) usually associated with other abnormalities as diabetes mellitus, deafness, etc. According to OXPHOS hypothesis (Chapter 6) the gradual accumulation of mistakes in the mitochondrial genome may be also the cause of aging and age-related diseases.

8.3. POLYGENIC INHERITANCE

Most phenotypic traits (e.g. body height, IQ) are determined by collaboration of many genes and their interaction with environmental factors (e.g. nutrition, psychosocial influences). Quantitative assesment of such traits in large populations show continuous variation in form of normal or near-normal distribution curve (Fig. 8.1).

Monogenic diseases and health disturbances caused solely by external agents represent the two endpoints of a broad scale. Most of the common chronic diseases in this sense can be found somewhere in the middle area of this scale - that is they arise as a combination of a given genetic background (consisting of effects of many genes) and external agents promoting the manifestation of the disease (Tab. 8.6). In other words the genetic backround cocks and the external factor pulls the trigger of the pathogenesis.

The mode of inheritance of these diseases do not obey the Mendelian rules (although the inheritance of each gene does). The proportion of the genetic and environmental factors may vary even in the same disease. For example high cholesterol level can be a consequence of one gene (in familiar hypercholesterolemia - faulty LDL-receptor gene) or may arise as a combination of some background genes (regulating cholesterol metabolism and isoforms of apoproteins) and unhealthy life-style (Fig. 8.2).

Recently the controversial discussion about inheritance of intelligence and psychosocial aberrant behaviour were heated up. Some researcher claimed to find the gene(s) responsible for homosexuality, alcoholism, drug addiction, aggressive behavior and even marital infidelity. It is beyond any

Tab. 8.6. Chronic diseases arising as a consequence of genes and environmental factors

DISEASE	GENETIC FACTORS	ENVIRONMENTAL FACTORS
Coronary heart disease	LDL-receptor gene Other genes of cholesterol metabolism, platelet and endothel function, apoproteins	not necessary high caloric and fat intake, low proportion of PUFA lack of exercise oral contraceptives diabetes, hypertension
Obesity, NIDDM*, X-syndrome	Regulation of insulin secretion Insulin receptor Regulation of energetic metabolism Disturbances of neurotransmitters	high caloric and fructose lack of exercise psychological factors
IDDM*	HLA association (autoimmunity)	viral infection cow milk protein
Hypertension	Membrane transport systems renin-angiotensin system	salt intake alcohol
Lung cancer	Exists but not known	smoking
Breast cancer	BRCA1 gene on region 17q (breast Ca in young age)	hormone equilibrium total estrogen exposure? (early menarche and late menopause fat intake (questioned)
Allergy	IgE receptor gene defect (chromosome 11) cytokine genes controlling IgE production (Chr. 5)	allergens
Hip dislocation	Acetabular dysgenesis familial joint laxity	breech position at birth swaddling with extended legs

*IDDM, NIDDM = insulin and non-insulin dependent diabetes mellitus

Tab. 8.7. Genes, behavior and intelligence

DISORDER OR BEHAVIORAL PATTERN	BACKGROUND GENETIC DEFECT
Homosexuality (male)	Region Xq28, (near the end of the long arm of the X chromosome) not confirmed by others
Alcoholism	Dopamine receptor gene low serotonin production
Aggresion*	Monoamine oxidase gene
Gifted learners (rats)	high activity of protein kinase C

*Some women researchers claim that aggressivity is simply linked to the presence of Y chromosome

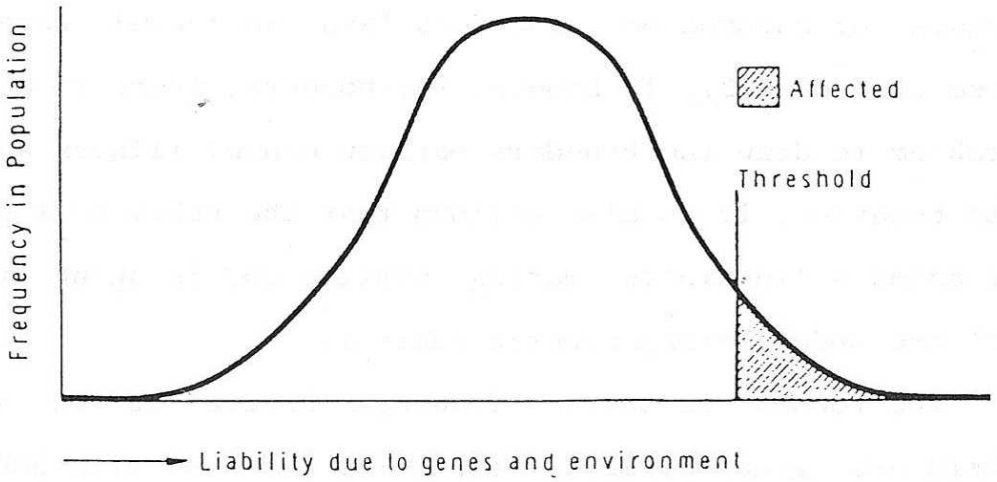

Fig. 8.1. Distribution of the values of a trait determined by many genes and environmental factors.

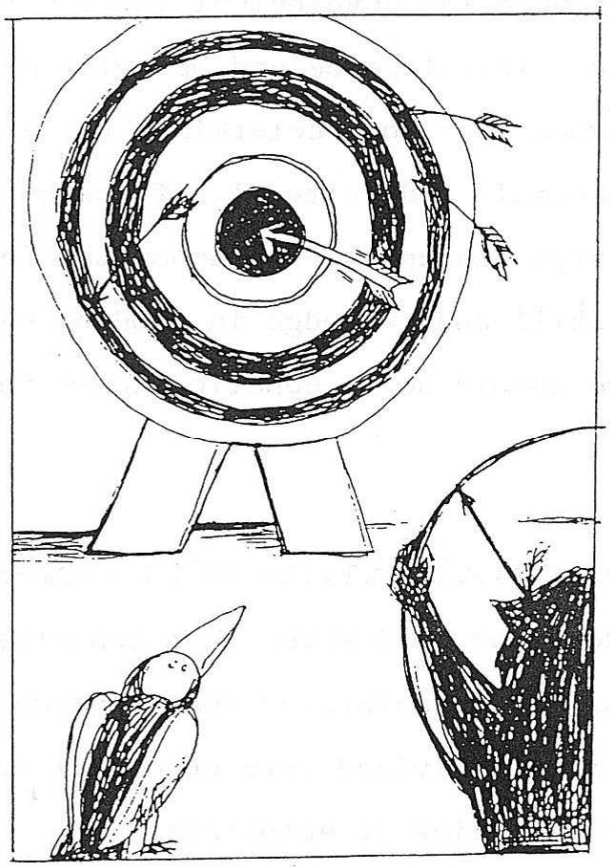

Fig. 8.2. A major gene (the arrow hitting the "10") has the same effect as the additive effect of some genes with small effect and the environmental factors (2+3+1+4=10).

doubt that distinct syndromes (e.g. dyslexia, Tourette's syndrome) may have defined genetic cause and some monogenic diseases or chromosome disorders lead to mental retardation (see Chapter 8.3). In humans, furthermore, there is a serious problem to draw the boundary between mental illness and altered behavior. It is also obvious that the behavioral patterns of animals (instincts, mating, hunting and foraging strategies) are under strong genetic control.

The recent scientific findings, however do not say that there are genes directly responsible for erratic behavioral patterns or IQ but they regulate the metabolism of certain neurotransmitters (Tab. 8.7). Like anything else in the human body, the structure and metabolism of the nervous system is based on genes which govern its development working in harmony with the environment. Intelligence and behavior are therefore strongly influenced but not determined by genes. The space left for psychosocial and cultural influences (family, education, etc.) is large enough to influence the individual outcome of behavior, skill and kwoledge in good or bad directions. In humans there should be no genetic excuse for wrongdoing.

8.4. CHROMOSOMAL ABERRATIONS

Chromosome disorders can be divided into two classes: **abnormalities of number and structure**. From practical reasons the first class is further divided into abnormalities of gonosome number and abnormalities of autosomes.

Abnormalities of chromosome number arise from nondisjunction during the meiosis, that is, from the failure of two homologous chromosomes during the first or second division of

the meiosis to pass to opposite poles of the cell (Fig. 8.3). Nondisjunction can also occur in early divisions of a zygote and this leads to chromosome mosaic (patients with 2 different caryotypic cell lines). Factors leading to non-disjunction are not completely clear. In trisomy of 21st chromosome the incidence of the disorder increases with the age of the mother.

GONOSOMAL NUMERICAL ABERRATIONS

If the nondisjunction affects the X chromosomes of an oocyte, the result will be a cell with caryotype 24,XX and a second with 22,0. After fertilization with normal spermatozoa (23,X or 23,Y) the following combinations arise:

47,XXY; 47,XXX; 45,Y; 45,X

Three out of these four combinations are compatible with life and manifest itself as distinct syndromes (Tab. 8.8). Life without X chromosome (45,Y) is impossible. Nondisjunction can also occur during spermatogenesis (spermatozoa 24,XY and 22,0) which after fertilizing a normal ovum give zygotas 47,XXY or 45,X.

ABNORMALITIES IN NUMBER OF AUTOSOMES

There are only some autosomal trisomies compatible with life, and only one (Down's syndrome) where the affected person can reach the phase of adulthood (Tab. 8.9). The trisomy of an extra chromosome in this disease was discovered in 1959 by Lejeune and coworkers. It is interesting that the activity of the enzyme superoxide dismutase (Chapter 6) coded on the chromosome 21 is elevated by approximately 50 %, what corresponds to the number of the genes (3 instead of 2).

In some cases the affected children have another caryotype and the syndrome is caused by translocation of a third

chromosome 21 to chromosome 14. The story actually begins in mother, who has only 45 chromosomes. The 46th, one of the two chromosomes 21 is translocated to chromosome 14. The mother despite abnormal caryotype has normal amount of genetic material and is clinically healthy. Her oocytes (Fig. 8.4) are of two types:

1/ 22,X -21 and

2/ 23,X with an extra 21 translocated to 14

Fertilization with normal spermatozoa leads to

1/ 21 monosomy (antimongolismus: 45,XX -21 or 45,XY -21)

2/ 46,XX or 46,XY with the translocated chromosome 21/14 and clinical signs of Down's syndrome.

Other types of autosomal aneuploidia (abnormal number of chromosomes) have been reported among spontaneously aborted fetuses - there are obviously not compatible with development and life. In somatic cells, however, (e.g. in liver) aneuploidia and polyploidia is not uncommon, but this does not affect the function of the organ and is not transmitted to the next generation.

ABNORMALITIES OF CHROMOSOME STRUCTURE

These result from chromosome breakage and reunion. The structural rearrangement of the genetic material may be **balanced** (no change in the amount of genetic material in the cell) or **unbalanced** (loss or gain of chromosome material). The most frequent changes are **translocations, deletions and inversions**. The above described second possibility of Down's syndrome is a special case of translocation (Robertsonian translocation).

An example of unbalanced change is the deletion of the short arm of chromosome 5 leading to **cri du chat** syndrome.

The affected children exhibit mental and physical retardation and their cry resembles the mewing of cats. Other deletions were described, too; mental and physical retardation and multiple malformations are common in these syndromes.

Some chromosomal aberrations are connected with the occurence of leukemias, lymphomas and solid malignant tumors. One of such examples is the occurence of **Philadelphia** chromosome (actually chromosome 21 without half of its long arm) in chronic myeloid leukemia. Translocations can also lead to malignancies - probably through placing cellular oncogenes on wrong place.

FRAGILE X CHROMOSOME SYNDROME

This unusual chromosome disorder was discovered in the 1970s. Investigated under electron microscope the tip of the long arm of the X chromosome seems to be connected to the rest of the chromosome by a thin tread. This site is very fragile and the chromosome may be easily broken. The affected children are mentally retarded and display macrorchismus. The inheritance of this syndrome partly obeys the rule for X-linked inheritance but 20 - 50 % of the affected males are asymptomatic. They pass the defect to their daughters and in the children of these women (who are asymptomatic, too) the size of the fragile site tends to increase, sometimes containing more than twenty times as much genetic material as int their mothers. From biochemical point of view these fragile sites in affected persons contain more methyl groups attached to DNA than those of normal individuals or asymptomatic carriers of the trait.

Broken chromosomes (1-2 %) can be seen even in caryotypes of healthy individuals. This number is increased in persons

exposed to damaging environmental factors (radiation, toxicants, etc.). Fragility of chromosomes in Fanconi's anemia and Bloom's syndrome is probably caused by genetic defects of DNA and chromosome repair systems.

Tab. 8.8. Sex chromosome abnormalities

CARYOTYPE	NAME	SEX	DESCRIPTION
47,XXY	Klineferter's sy.	male	tall, eunuchoid males, hypogonadism feminine distribution of hair, often low IQ, psychosocial problems
47,XYY	"supermale"	male	tall but often without phenotypic abnormalities, may have psychosocial problems
45,X	Turner's sy	female	short stature, ovarian dysgenesis, webbing of the neck, often heart, skeletal and renal abnormalities
47,XXX	"superfemale"	female	extremely variable, often without phenotypic abnormalities. Low IQ, psychosocial problems. Often fertile.

Tab. 8.9. Autosomal trisomies compatible with life

TRISOMY	NAME	DESCRIPTION
21	Down's sy. mongolism	Broad flat face, slanting eyes, epicanthus short nose, big tongue, arhed palate, dental anomalies, dysplastic ears; congenital heart disease, megcolon; short and broad hands, widely spaced big toes; mental retardation and growth failure
18	Edward's sy.	Low birth weight, severe mental retardation; facial abnormalities - micrognathia, malformed ears, cleft lip and palate, malformations of fingers. Survival for a few months.
13	Patau's sy.	Low birth weight, severe mental retardation; facial abnormalities - broad nose, mirophtalmia, frequent polydactyly, syndactyly. Survival is very short.

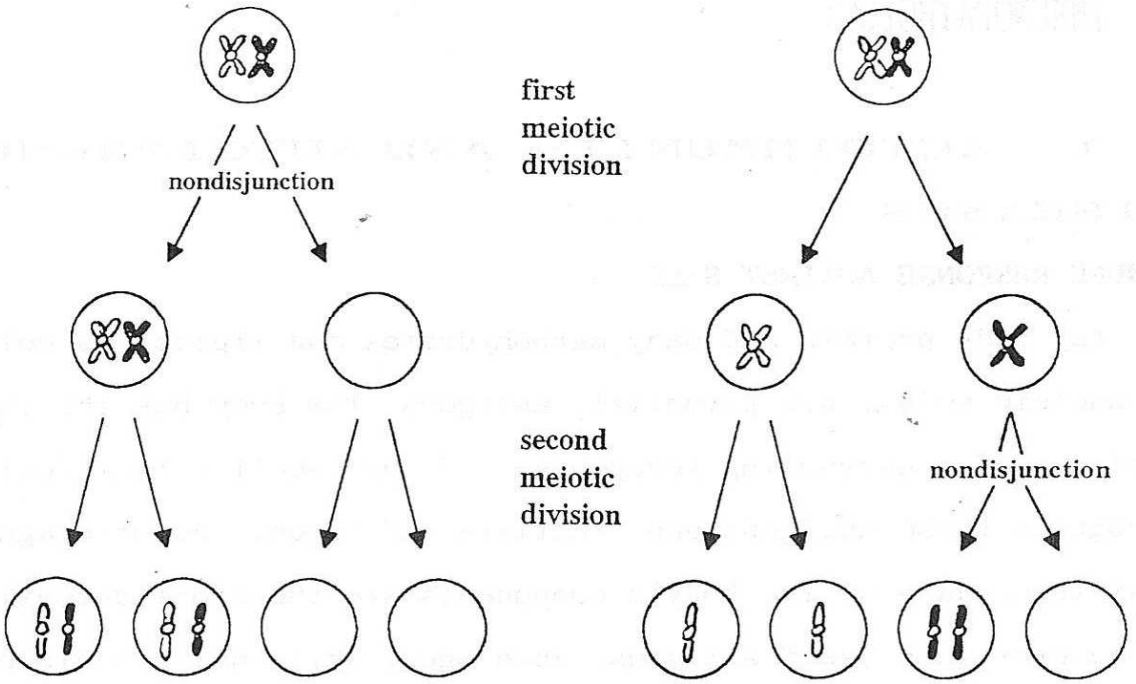

Fig. 8.3. Two possibilities of nondisjunction in oocytes.

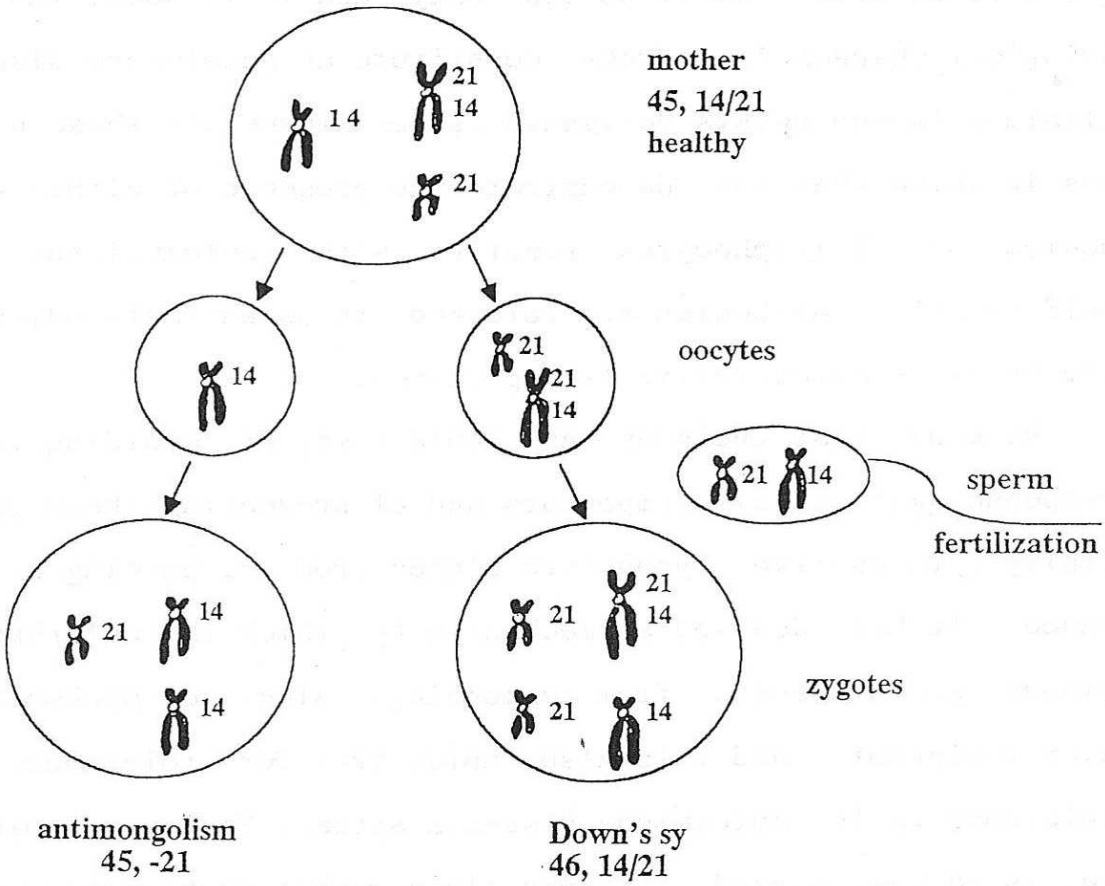

Fig. 8.4. The mechanisms of production of Down's syndrome through translocation of chromosome 21 to chromosome 14 in the mother.

9. IMMUNOPATHOLOGY

9.1. AUTOIMMUNITY AND AUTOIMMUNE DISEASES

IMMUNE RESPONSE AGAINST SELF

Any body protein and many carbohydrates and lipids, as well as nucleic acids, are potential antigens. The body has the capability of constructing receptors - T- or B-cell - that could recognize these antigens and initiate and immune response against them. Many of the body's components are therefore potential autoantigens (self antigens, autologous antigens) capable of inducing autoimmunity, humoral or cellular. Hence, autoimmunity is an immune response against components of self. It may or may not have adverse effects on the body, and if it does, the pathological changes it induces constitute an autoimmune disease. Clinical immunologists designate as autoimmune all those diseases in which they can demonstrate the presence of either antibodies or T-lymphocytes reactive with autoantigens. The self-reactive antibodies are referred to as autoantibodies and the cells as autoreactive T-lymphocytes.

We know that the body has found a way of avoiding immune response against self components and of preventing these potentially autoreactive lymphocyte clones from responding - tolerance. It has devised a mechanism by which the autoreactive clones are prevented from responding. Alas, no mechanism is ever foolproof, and this also holds true for tolerance. When tolerance fails, autoimmune diseases appear. Moreover, tolerance, as we have already learned, is a rather problematic mechanism in the sense that even in a healthy individual there are

always some autoreactive lymphocyte clones present. Autoreactive B-lymphocyte clones are so common that some immunologists question the existence of B-cell tolerance altogether. Some degree of autoreactivity is therefore part of the normal physiology of the immune system: autoimmunity is part of general immunity (or immunopathology). The physiological autoimmunity does no harm to the body and may even be beneficial to some extent. It is only when an exaggerated autoimmune response develops that a disease may ensue. The exaggeration is not part of the normal physiology of the immune system, it is a pathological event.

MECHANISMS OF AUTOIMMUNITY

It is not known why, on rare occasions, the immune response derails and mounts an attack on self components. This lack of knowledge is attributable to the fact that it is not yet understood how tolerance is accomplished. Once the problem of tolerance induction is solved, much will also become clear about autoimmunity. At this stage, however, the three main hypotheses of tolerance must be considered as possible explanations of autoimmunity.

The first hypothesis is that autoreactive lymphocyte clones (mainly T but also B) are functionally deleted or inactivated by the encounter with autoantigens in the early stages of development. Autoimmunity ensues when some of the clones are not, for whatever reason, deleted. The normally forbidden clones may then attack the tissues of the body. The original explanation for the emergence of forbidden clones was that they resulted from somatic mutations in the receptor genes. The mutations were thought to alter the antigen receptor in such a way that it

aquired high affinity for a particular autoantigen. Numerous experiments have shown, however, that autoimmune disease can be induced in animals at will. The potential for autoimmune response is apparently present all the time, the response does not have to wait for the occurrence of a rare constellation of mutations which alter the specificity of an existing clone. An alternative to the mutation hypothesis is to postulate that certain autoreactive lymphocyte clones are not eliminated during the tolerance-induction process. If the clones are always present, the question is what awakes them from their dormancy? This question will be returned to later.

The second hypothesis of tolerance postulates the existence of antigen-specific suppressor T-lymphocytes. If such cells were to exist, their failure to suppress clones specific for self antigens could result in autoimmunity and in autoimmune disease. The failure would have to pertain to only a few clones and the suppression in normal tolerance would have to be antigen-specific because individuals with autoimmune diseases do not show an exaggerated immune response to all non-self antigens. The existence of antigen-specific suppress- or T-cells, however, remains very much in doubt and the evidence for tolerance as caused by suppression is unconvincing.

Finally, the third main hypothesis of tolerance invokes the highly controversial postulate of idiotype/antiidiotype network. If response to self components were prevented by some kind of balancing act within the network, autoimmunity could develop from a disturbance in this balance. As already mentioned, of the three hypotheses, the network hypothesis is supported least by experimental evidence. Although spontaneous or in-

duced emergence of idiotype-specific antibodies has been documented and although such antibodies (and, for that matter, T lymphocytes as well), may be responsible for or contribute to certain autoimmune diseases, network disturbances as a cause of such diseases present an improbable proposition.

Be that as it may, autoimmune disease only occurs when tolerance is somehow lifted. One possible explanation of the way in which tolerance is overcome is molecular mimicry, it is the only explanation for which there is compelling evidence. The main postulate of the molecular mimicry hypothesis is that the microbes try to escape recognition by mimicking self antigens. If, however, the self and nonself epitopes were similar but not identical, the host could respond immunologically to the microbial epitopes and the response could then break immunological tolerance and lead to an assault on the host tissue. The microbial agent might be cleared, but the immune response that it initiated would continue and develop into an autoimmune disease. The process might become self-perpetuating if the attack on the tissue releases self antigens, thereby further stimulating the response.

Sharing of epitopes between infectious agents and host-cell proteins has been amply documented. In one study, for example, nearly 4 % of monoclonal antibodies raised against a large panel of DNA and RNA viruses were found to cross-react with host epitopes expressed on uninfected tissues. Furthermore, a possible involvement of the shared epitopes in diseases pathogenicity has been indicated. For example, a neutralizing monoclonal antibody directed against an antigen of Coxsackie B4 virus also reacts with heart muscle, and intris-

tingly, this virus is frequently found in patients with inflammatory disease of the heart muscle. More direct evidence has been provided by studies in which a peptide of the hepatitis B virus polymerase was shown to induce an autoimmune response leading to tissue injury, similar to that induced by a self protein, the myelin basic protein, with which the peptide shares an epitope. In another study, the protein gliadin, a dietary component of the wheat gluten, has been shown to share epitopes with the E18 protein of human adenovirus Ad-12. Interesttingly, A-gliadin is believed to active the coeliac disease characterized by injury of the small intestine which the adenoviruses inhabit. Moreover, almost 90% of patients with coeliac disease (compared to 17 % of control patients) show evidence of infection by Ad-12, but not by the closely related virus Ad-18, or other intestinal viruses. In yet another study, the hexapeptide QTDRED has been found to be shared by HLA-B27 and Klebsiella pneumoniae nitrogenase. The bacterium has been implicated by some investigators in the aetiology of ankylosing spondylitis, a disease known to occur more frequently in HLA-B27 individuals than in individuals bearing other HLA-B alleles. Moreover, sera from a significant proportion of HLA-B27 patients with ankylosing spondylitis but not sera from control patients reacted with the hexapeptide. Some of the known short sequence similarities between microbial and host proteins are listed in Tab. 9.1. The evidence available suggest that some of these similarities may be factors contributing to the development of autoimmune disease.

THE CAUSES OF AUTOIMMUNE DISEASES

Whatever the mechanism by which exaggerated autoimmune res-

ponse develops, some abnormal circumstances that lead to this exaggeration must exist. What might these ciercumstances be?

Despite their great diversity, the individual autoimmune diseases have certain features in common that may be taken as clues when considering what exactly goes awry when an autoimmune condition arises.

Tab. 9.1. Sequence similarities between microbial proteins and human host proteins. Immunological cross-reactivity between proteins in each pair has been demonstrated. Peptide sequences are expressed in the single-letter code.

Protein	Residue	Sequence
Human cytomegalovirus IE2	79	PDPLGRPDED
HLA-DR molecule	60	VTELGRPDAE
Poliovirus VP2	70	STTKESRGTT
Acetylcholine receptor	176	TVIKESRGTK
Papilloma virus E2	76	SLHLESLKDS
Insulin receptor	66	VYGLESLKDL
Rabies virus glycoprotein	147	TKESLVIIS
Insulin receptor	764	NKESLVISE
Klebsiella pneumoniae nitrogenase	186	SRQTDREDE
HLA-B27 molecule	70	KAQTDREDL
Adenovirus 12 E1B	384	LRRGMFRPSQCN
alfa-gliadin	206	LGQGSFRPSQQN
Human immunodeficiency virus p24	160	GVETTTPS
Human IgG constant region	466	GVETTTPS
Measles virus P3	13	LECIRALK
Corticotropin	18	LECIRACK
Measles virus P3	31	EISDNLGQE
Myelin basic protein	61	EISFKLGQE

The first common feature is that a multiplicity of factors must conspire to turn the immune system against the body's own tissue. At the top of the list of these factors we must place

genetic predisposition, for which there is evidence in all the diseases examined. The pattern of inheritance is always complex, indicating that several genes are involved. It is unlikely that the genes are the same for the different diseases, although one gene complex - the Mhc - has consistently been implicated in the control (Tab. 9.2). Granting that the intensity with which this complex has been studied may have contributed to the consistency of this finding, the known involvement of the Mhc in the control of the immune response nevertheless suggests that it may, indeed, play a major part. We have almost no knowledge of the other genes involved in the induction of autoimmunity. It should be emphasized, however, that genes merely predispose a person to the disease, the disease does not develop unless the other factors play their part.

The second group of factors consists of the various exogenous agents that may well be the moving force behind the development of the disease. Viruses are the most likely candidates for this role and viral aetiology is indeed suspected in many of the diseases. However, solid evidence implicating viruses is still lacking. In some instances, the factors may not be truly exogenous, but somewhere between self and nonself, as in the case of some persistent infections caused by endogenous viruses. The exogenous factors may be responsible for the persistance of the stimulus and consequently for the chronicity of the disease.

The self components themselves make up the third group of factors involved in the induction of autoimmunity. They are probably not, as was once believed, the primary inducers of the disease, but they are certainly its prime target. There are

a variety of ways in which these molecules may become immunogenic to the body's immune system, they may be modified (for example, by drugs), or be degraded into immunogenic components, or simply exposed in their hiding-places inside the cell or inside an organ normally inaccessible to lymphocytes.

Finally, the fourth group contains the physiological factors. Since many autoimmune conditions are diseases of ageing individuals, there must be some changes associated with the ageing processs that are responsible for the malfunction of the immune system. The prevalence of many autoimmune diseases in one of the sexes (female) suggests hormones may be among the contributing factors.

Tab. 9.2. Selected human autoimmune diseases and their association with HLA haplotypes

Disease	Target	association with HLA haplotype
Systemic lupus erythematosus	Connective and vascular tissues	DR3
Rheumatioid arthritis	Connective and vascular tissues	DR4
Mixed connective tissue disease	Connective tissue	B7, Dw1
Scleroderma	Connective tissue of the skin	B8?
Sjogren's(sicca)syndrome	Salivary and lacrimal glands	DR3
Autoimmune thyroiditis	Thyroid gland	DR5
Graves'disease	Thyroid gland	DR3
Addison's disease	Adrenal gland	DR3
Insulin-dependent diabetes mellitus	Pancreas	DR3, DR4, DR2*
Pernicious anaemia	Stomach	DR5
Atrophic gastritis	Stomach	?
Coeliac disease	Small intestine	DR3, DR7
Inflammatory bowed disease	Intestine	B27
Chronic active hepatitis	Liver	B8?
Primary biliary cirrhosis	Liver	?
Primary glomerulonephritis	Kidney	C4B* 2.9
Idiopathic membranous nephropathy	Kidney	DR3

Acute anterior uveitis	Eye	B27
Ocular cicatricial pemphigoid	Eye	B12
Scleritis	Eye	B15?
Multiple sclerosis	CNS	DR2
Demyelinating neuropathies	CNS	?
Rheumatic fever	Heart	?
Myocarditis	Heart	?
Pemphigus vulgaris	Skin	DR4
Bullous pemphigoid	Skin	A24?
Herpes gestationis	Skin	?
Autoimmune haemolytic anaemia	Blood (erythrocytes)	?
Idiopathic thrombocytopenia purpura	Blood	DR2?
Autoimmune neutropenia	Blood (neutrophils)	?
Autoimmune disease of the testis	Testis	?
Myasthenia gravis	Muscle	DR3
Myositis	Muscle	?

CNS - central nervous system; * - resistance haplotype

The main problem in studying autoimmunity is how to arrange the individual factors into a causal chain. What comes first and what follows? What is the cause and what the consequence? Probably, there is no single causal chain, but a complex network of interactions. To identify this network may be one of immunology's most challenging tasks.

CAUSE OF TISSUE DAMAGE

Autoimmunity develops into a disease when the components of the immune system begin to damage the body. The following components have been implicated as the cause of damage in at least some of the diseases: antibodies, complement, antigen-antibody complexes, T-lymphocytes, macrophages, natural killer cells, and K cells.

Antibodies have been incriminated in the pathology of a number of autoimmune diseases. Some of the diseases can be transferred by antibodies to a healthy individual either transplacentally or by serum inoculations in experimental situati-

ons. The level of autoantibodies, however, often does not correlate with the extent of tissue damage, indicating that other factors must also contribute. Although in some diseases cell-bound antibodies may act by interfering with the tissue's normal functions, more often than not their damaging effect may actually be mediated by complement. Antibodies bound to cell-surface autoantigens activate the classical pathway of the complement cascade and the activated complement components then damage the tissue either directly, by punching holes into the plasma membrane, or indirectly, by initiating an inflammatory response. Depletion of serum complement is known to accompany some autoimmune diseases.

If the autoantigen is a soluble molecule, antibodies may bind to it in the body fluids and form immune complexes which may circulate and be deposited at sites distant from the actual target of the autoimmune response. Like cell-bound antibodies function of an organ or they may fix complement which then damages the tissue directly or indirectly.

T-lymphocytes have been strongly implicated in the pathogenesis of certain autoimmune diseases by the demonstration that such diseases can be induced in healthy individuals into which T-cells of a diseased individual have been inoculated. Successful transfers of autoimmune diseases with T-lymphocyte clones specific for a particular antigen have also been reported. Both T_c- and T_h-lymphocytes have been shown to cause tissue damage. The T_c-cells presumably attack the target tissue directly, the T_h-cells act by providing help to T_c-cells, but also by releasing cytotoxic lymphokines and by contributing to a developing inflammatory response.

Macrophages may contribute to autoimmune tissue lesions when they are activated by lymphokines or when they become "armed" by cytophilic antibodies. They may then become cytotoxic. The evidence for the participation of natural killer and K cells in the autoimmune attack on tissues is circumstantial at best, and the cells are usually not found in large numbers in the tissue lesions. The main human autoimmune diseases are listed in Tab. 9.2.

9.2. IMMUNODEFICIENCY DISEASES

In this chapter we shall ask ourselves what happens when the specific immune system fails. The answer to this question has been provided by the unfortunate patients who suffer from various disturbances in the development of their lymphoid cells - the immunodeficiency diseases (some authors extend this designation to any defect of the immune system, whether it concerns lymphocytes, granulocytes, the complement system, etc.). The first immunodeficiency disease was described in 1952 by Ogden C. Bruton and since then, hundreds of other cases have been reported. Whether any two of these cases are exactly the same is questionable, but there are enough similarities among them to allow their grouping into individual diseases.

The defect may affect T-lymphocytes, B-lymphocytes, or both. It may occur in different stages of stem-cell, T-cell, or B-cell differentiation, the deficiency may also be secondary to an embryological abnormality. It may be congenital, caused by a mutation in a gene, or it can be acquired, for example, through a virus infection. The defect may be fatal or not, depending on the stage of stem cell or lymphocyte differentiation

at which it occurs.

A brief description of the major immunodeficiency diseases follows.

SEVERE COMBINED IMMUNE DEFICIENCY

Severe combined immune deficiency (SCID) is the most extreme form of immunodeficiency disease that affects both T and B lymphocytes (hence the adjective "combined"). It is a disorder of infants and young children, individuals suffering from it do not live beyond early childhood. The characteristic manifestations of the disease include severe infections in the first 2 months of life, failure to thrive, chronic diarrrhoea, recurrent pneumonia, candidiasis of the skin, oesophagus, and oropharynx (part of the pharynx between the soft palate and the upper edge of the epiglotis), and chronic recurrent inflammation of the middle ear. The patients are susceptible to all kinds of pathogens, but they are particularly likely to be infected with the cytomegalovirus, varicella, Candida albicans, and Pneumocystis carrinii. These are infections in which T-lymphocytes are believed to have a dominant protective function. The children may be protected against other microorganisms by antibodies that they acquired from their mothers transplacentally or with milk. Lymph nodes, tonsils, and the thymus are either absent in SCID patients or are very small. Immunoglobulins are at a low level or absent. T-lymphocytes are always absent but B-lymphocytes may be present in normal numbers in some cases. The disorder is believed to be caused by a defect in the pluripotent lymphoid stem cell (Fig. 9.1), but it may occur at different stages of differentiation and give rise to a variety of conditions that bear separate designations.

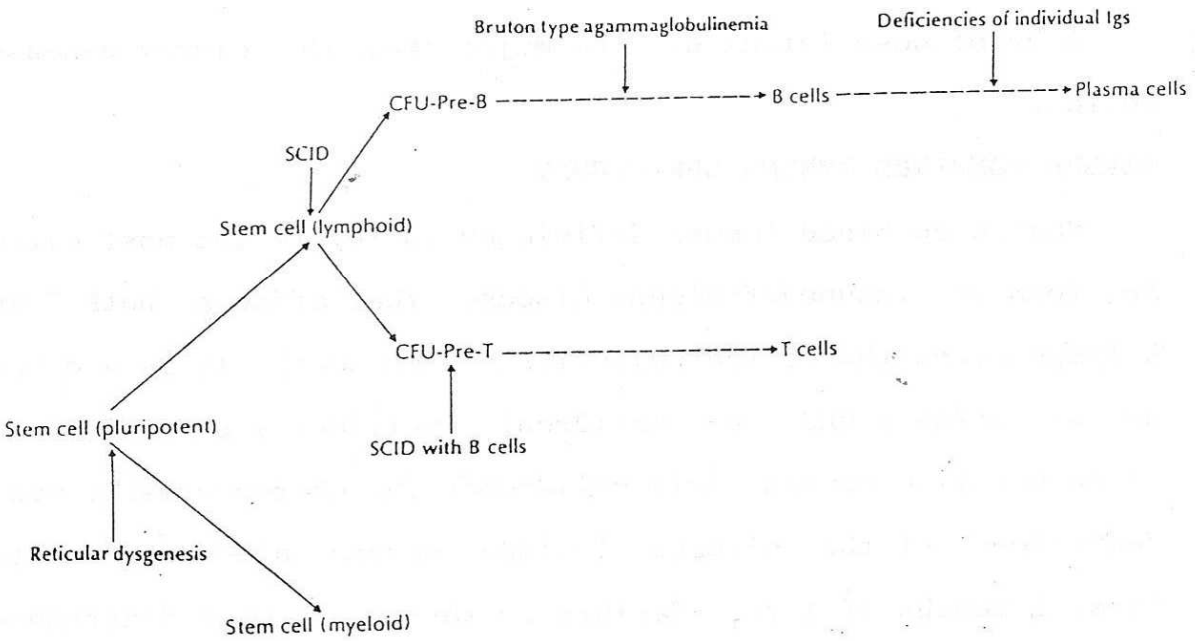

Fig. 9.1. Stages in the lymphocyte differentiation affected in immunodeficiencies. CFU - colony-forming unit, Igs - immunoglobulins, SCID - severe combined immune deficiencies.

Reticular dysgenesis (Gk dys, bad, genesis, development) is a rare disease, characterized by the total, or almost total, absence of T- and B-lymphocytes, granulocytes, and monocytes, erythrocytes and thrombocytes are present. The defect responsible for this disease must, therefore, occur at the level of a stem cell common to the lymphocytic and myeloid lineages (Fig. 9.1). Children suffering from reticular dysgenesis die shortly after birth.

Swiss - type agammaglobulinaemia, first described by the Sviss physician W.H.Hitzig in a Swiss patient, is characterized by a drastic reduction of the gamma-globulin level in the

patient's serum. The defect is inherited as an autosomal recessive trait and results in the abnsence of both T- and B-lymphocytes. The fact that patients have normal numbers of other blood cells, including granulocytes, indicates that the lesions occur at the level of a stem cell common to the T- and B-lymphocytic lineages, one or more steps away from the stem cell affected in reticular dysgenesis.

SCID with ADA deficiency resembles Swiss-type agammaglobulinaemia but differs from it in that it often leads to bone abnormalities and is associated with an identiable enzyme defect. The afflicted enzyme is adenosine deaminase (ADA), which catalyses the conversion of adenosine to inosine (Fig. 9.2). ADA deficient patients carry a homozygous mutations at the ADA structural locus on chromosome 20. The heterozygotes have half the normal ADA level, but they remain clinically normal, the homozygotes lack the enzyme completely. ADA deficiency also occurs without an immunodeficiency, and vice versa. For example, in the Kung tribe living in the Kalahari desert, the defactive ADA gene is present without SCID in 1 % of the population.

ADA deficiency with or without immunodeficiency is also relatively frequent in Arabian horses. Some immunologists speculate that the accumulation of intracellular adenosine caused by the absence of ADA leads to the inhibition of pyrimidine synthesis (pyrimidine starvation) and to cell death. Others believe that the accumulation of adenosine leads to an increase of cyclic AMP, a molecule believed to inhibit many lymphocyte functions. ADA is indeed found in lymphocytes, but it also occurs in erythrocytes and other cells.

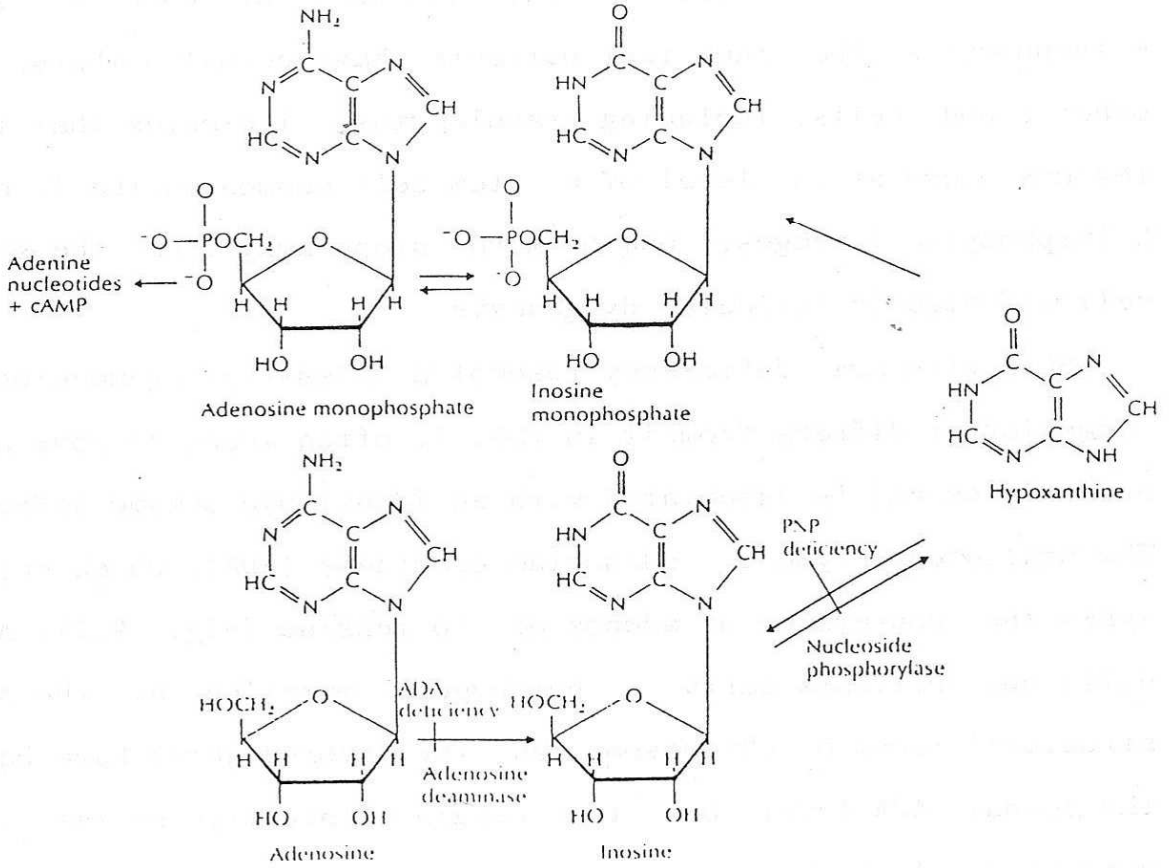

Fig. 9.2. Adenosine metabolism and sites on the pathway in which metabolic blocks result in adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiencies.

Immunodeficiency with short - limbed dwarfism occurs in three versions, of which only type I is associated with SCID, type II is associated with T-cell immunodeficiency and type III with B-cell immunodeficiency. All three types are characterized by short, pudgy extremities, while the head is of normal size, which distinguishes this condition from achondroplasia - a hereditary, congenital disturbance of cartilage-forming cells at

bone ends leading to dwarfism with short limbs and normal trunk. Another characteristic is the development of redundant skin folds around the neck, and large joints of the extremities. Type I patients die early in infancy, type II and type III patients may survive to reach the second to fifth decade of their lives. Types II patients may also have cartilage-hair hypoplasia leading to light, thin, and sparse hair, as well as to cartilage abnormalities.

SCID with B-lymphocytes differs from the previous two SCID diseases in that it affects only T, and neither B nor any other blood cells. The lesion apparently occurs one step further away from the stem cell, perhaps at the level of the T-progenitor cell. Although B cells are present, the patient's serum contains only low levels of immunoglobulin - some IgM but only very little IgG and IgA. The reason for the Ig deficiency is apparently the unavailability of T-cell help which is necessary for the differentiation of B lymphocytes into Ig-secreting plasma cells. Supporting this conclusion is the observation that when the patient's B-lymphocytes are cultured in vitro in the presence of normal T-lymphocytes, they synthesize Ig normally.

The SCID defect can be corrected by bone marrow transplantation. If the donor of the graft is a histocompatible sibling, the cure rate approaches 70 %. Transplantation of totally HLA mismatched adult bone marrow results in a universal failure, with most of the recipients dying of severe graft-versus-host disease. Slightly more successful are transplants of haemopoietic tissue from fetal donors after the removal of mature T-lymphocytes from the graft, and with concominant drug therapy. Transplantation of thymic epithelium prepared by culturing

thymus fragments in vitro has also been reported to have some degree of success. The enzyme defect in ADA deficiencies can be partially corrected by transfusion of erythrocytes from healthy individuals.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

The syndrome is believed to be caused by the human immunodeficiency virus - 1 (HIV-1), a member of the subfamily Lentivirae and of the family Retroviridae. About 30 % of the individuals exposed to the HIV-1 never develop any symptoms of disease (Fig. 9.3). This resistance is probably influenced by a number of factors, in the first place the dose of the virus. As the virus is not very infective, exposure to a relatively large dose of the virus is required for a successful infection.

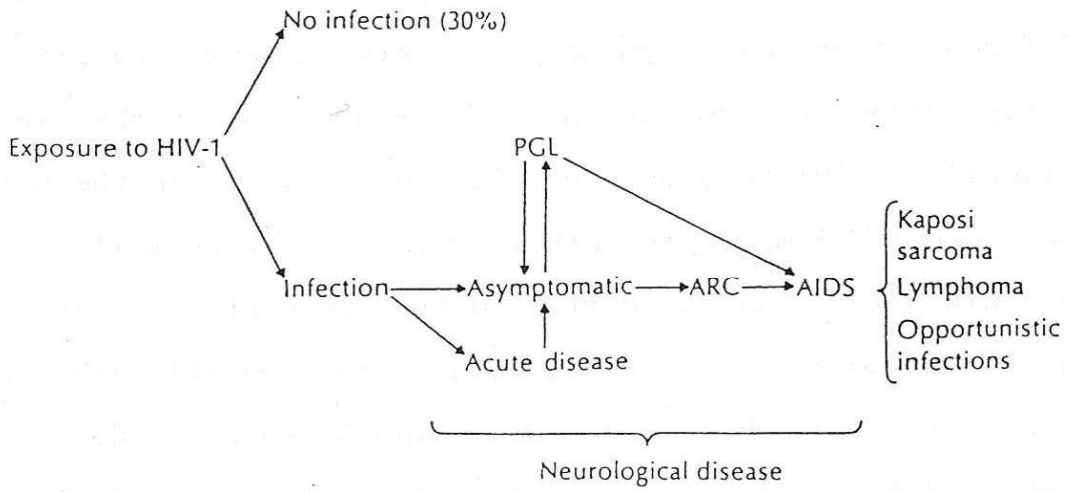

Fig. 9.3. Clinical course of an HIV-1 infection. AIDS - acquired immunodeficiency syndrome, ARC - AIDS-related complex, PGL - persistent generalized lymphadenopathy.

Another factor is probably the immune response. Among the infected individuals identified through a seroconversion (the appearence of antibodies specific for the glycoprotein 120, gp 120, of the viral envelope) only 10-20 % show signs of an acute infection, including fever, rash, enlarged lymph nodes, a feeling of tiredness, waking up at night, sweating, and headaches - like a mild case of flu. Some of these patients, aswell as some of those who were initially without symptoms, develop persistent generalized lymphadenopathy (PGL). Some patients with PGL revert to the asymptomatic state, but both the asymptomatic and the PGL patients ultimately progress to the AIDS - related complex (ARC), characterized by lasting high fever, weight loss, severe persistent diarrhoea, fungus growth in the mouth and throat, multidermatomal herpes zoster, hairy leucoplakia, persistent cough, and increasing difficulty in breathing. Most ARC patients, as well as increasing number of asymptomatic carriers and PGL patients develop a full-blown acquired immunodeficiency syndrome (AIDS) characterized by lymphopenia, marked decrease in the number of T_h-cells, opportunistic infections, and appearence of certain types of tumours (Kaposi sarcoma, carcinoma of the mouth and rectum in infected homosexuals, and B-cell lymphomas). Regardless of the presence or absence of AIDS or ARC, the HIV-1-infected individuals may develop neurological disorders characterized by encephalopathy (brain damage), myelopathy (disturbance of the spinal cord), and peripheral neuropathy (disease involving spinal cord or cranial nerves). These disorders are, however, most common and most severe among the patients with the most severe immune defects. The manifestations of these neurological disorders include

epilepsy-like seizures, increasing difficulty in thinking and concentration, a slowing of speech movements, loss of memory, depression, and progressive loss of overall mental abilities. Neurologic symptoms occur in some 60 % of AIDS patients but in 80-90 % of the patients neuropathological changes are revealed on autopsies. The average incubation period of AIDS is 28 months in adults and 8 months in children. The rate of progression of the disease is influenced by the occurrence of other sexually transmitted infections, infection with cytomegalovirus, administration of high doses of corticosteroids, pregnancy, and age(the rate is very rapid in newborns). At least some of these factors have a common denominator in that they involve exposure to many new antigens and thus presumably lead to activation of $CD4^+$ T-cells. Close to 90 % of all AIDS patients die within 2 years after diagnosis of the disease.

At the origin of most of these symptoms is the infection of cells in the immune system. The main defect in AIDS is the depletion of the $CD4^+$ T-lymphocytes, which of course have a central function in the immune response as helper cells. Secondarily, this defect compromises the function of other cells (T_C-lymphocytes, B-lymphocytes and macrophages). The depletion occurs progressively with time so that at any one moment virus replication can be detected in 10^{-3} to 10^{-4} of lymphocytes (or 0.01 % of the circulatory lymphocytes). Even if $CD4^+$ T-lymphocytes are present, as in asymptomatic individuals and PGL patients, their proliferative capability is impaired by some intrinsic defect. Some of the possible explanations of this impairment include the selective loss of a subset of $CD4^+$ T-cells, suppression by viral proteins, and impairment of anti-

gen presentation by APC. The loss of functional $CD4^+$ T-cells makes the patient vulnerable to a variety of infections.

The infection of monocytes and macrophages leads to an enhanced release of interleukin-1 and tumour necrosis factor, two substances that act as endogenous pyrogens and are thus responsible for the chronic fever of AIDS patients. The TNF is a potent catabolic agent which is responsible for the pathogenesis of AIDS-associated cachexia (wasting). Infected monocytes and macrophages are probably also largely responsible for the neurological abnormalities. One immunologist compared them to a Trojan horse brought into the central nervous system across the blood-brain barrier, and with it the enemy, the virus. Activated macrophages release monokines and proteolytic enzymes that are toxic to the neural cells. Some of the released factors act as chemotactic agents that initiate an inflammatory response during which many innocent bystander cells, such as neurons and glial cells, are killed. Other factors may cause changes in vascular permeability and thus disturbances in the blood-brain barrier. The gp10 released from the infected cells may compete with neuroleukin and inhibit the growth of neurons (the two proteins show moderate similarity in their amino acid sequence). There is also some evidence that HIV can infect glial cells and astrocytes, which may express CD4 molecules. The infection may lead to demyelization and cell death.

Infection of B lymphocytes may lead to polyclonal activation, the generation of high immunoglobulin levels, and at the same time to poor antibody response against specific antigens. (polyclonal b-cell activation ensues in vitro when HIV extracts are added to B lymphocytes, perhaps because of the sequence si-

milarity between gp 120 and neuroleukin, a known B-cell activator). Disturbances in B-lymphocyte function may lower the resistance to pyogenic pathogens such as S. pneumoniae and H. influenzae, especially in children with AIDS. The high levels of nonspecific immunoglobulin may initiate autoimmune processes such as thrombocytopenia. For unknown reasons, antibody responses to polysacharide antigens are impaired in most PGL patients, including those with a normal $CD4^+$ T-cell count and normal antiprotein responses. The lowered immune response may predispose the AIDS patients to the development of certain types of tumour, in particular Kaposi sarcoma. Named after Moritz Kaposi, Kaposi sarcoma is a rare type of cancer which, prior to the emergence of AIDS, was found primarily in elderly men of Italian or Jewish origin. It apparently arises from malignant transformation of endothelial cells lining blood vessels in the skin of the extremities. It is characterized histologically by the appaerance of spindle cells, capillary-like spaces, and erythrocytes in the tissue. Outward signs of Kaposi sarcoma are dark-blue or purplebrown spots and nodules on the skin that look like birthmarks or bruises. The Kaposi sarcomas that are not associated with AIDS are mild, slow-growing cancers that become a serious hazard only 8-13 years after their diagnosis. The AIDS-associated Kaposi sarcomas are a different story: they can also occur in children and adolescents, they attack organs and glands, and they kill the patients within months. The observation that these cancers also appear in patients whose immune system has been suppressed by some other means indicates that the system is involved in their increased frequency in AIDS patients.

Another consequence of lowered immune response in the high incidence of opportunistic infections. Opportunistic pathogens are microorganisms that are commonly found in human without causing a disease. However, when the immune system of the host is compromised in some way, they become strongly pathogenic. The list of AIDS-associated opportunistic pathogens includes cytomegalovirus, herpes simplex virus, herpes zoster virus, C. albicans, Cryptococcus neoformans, Aspergillus, P. carrinii, Toxoplasma gondii, Cryptosporidium, M. avium intracellulare and M. tuberculosis. One of the most frequent AIDS-associated opportunistic pathogens is P. carrinii, a widespread but generally harmless protozoan. In compromised hosts, P. carrinii attacks alveolar macrophages causing desquamation and inflammation of the lung alveoli. The alveolar lumens become filled with large numbers of the protozoa and macrophages and this fact, together with the damage to the alveolar wall, causes pneumonia-like symptoms and difficulty in breathing. Why the opportunistic (rather than the nonopportunistic) pathogens are such a threat to AIDS patients is not known.

Persons infected with HIV-1 develop antibodies to many of the viral proteins, in particular the gp120. These antibodies, however, offer very little protection for at least two reasons. One reason is that the virus is unavailabale for immune recognition, but continously produced on activation of the infected cells. The persistence is assured by the integration of the virus into the hostcell chromosomes, infection of monocytes and macrophages, and the escape of the virus into the central nervous system in which it is immunologically inaccessible. The second reason is the variability of the virus, which is grea-

test in the gp120 component, the most likely target of an antibody attack. The gp120 contains regions of high variability, which alternate with relatively conserved regions in a manner reminiscent of antibody variable domains. An individual infected with HIV-1 may harbour several strains of the virus, which may differ in some of the total 9500 nucleotides making up the viral genome. All these strains seem to be derived from the original virus that infected the individual. Strains infecting two different individuals may differ by more than 1000 nucleotides, indicating that a given individual infected by one virus is rarely superinfected by another virus. How this "vaccination" against distantly related strains is achieved is not known. The variability of the virus in a single host is at least partly attributable to the fact that the reverse transcriptase has an error rate many orders of magnitude higher than eukaryotic DNA polymerases. The variability may provide the virus with an escape mechanism similar to that described for the influenza virus. The never-ending variation is one of the main obstacles to the development of a successful vaccine against HIV-1.

The virus does appear to induce protective cellular immunity, since HIV-1-specific T_c-lymphocytes have been found in healthy persons seropositive for HIV-1 and in AIDS patients after bone marrow transplantation. These cells, however, are of no use to the patient when the persistent infection destroys all the T_h-cells on which the T_c-lymphocytes depend functionally. Similarly, natural killer cells capable of killing HIV-1-infected target cells do develop but they, too, are strongly dependent on interleukin-2, normally provided by the T_h-lymphocytes.

DIGEORGE AND NEZELOF SYNDROMES

In 1965, Angelo M. DiGeorge described a child with abnormal facial characteristics (low-set and notched ears, "fish-shaped" mouth, shortened jaw, eyes set wide apart and slanted in the opposite direction to that seen in Mongoloids), innate heart disease, underdeveloped parathyroid gland, and underdeveloped thymus. This combination of defects is now referred to as the DiGeorge syndrome. The combination might seem at first to be purely coincidental, but a relatedness among the characteristics becomes obvious upon investigation.

What the four defects have in common is that before the twelfth week of gestation, the anlages of the affected organs (aortic arch near the heart, parathyroid gland, and thymus - all derived from the third and fourth pharyngeal pouches) and of the face all happen to be located in the same region of the embryo. One can therefore explain the fourfold defect as the result of an intrauterine accident damaging this region.

Infants with DiGeorge syndrome may die of heart failure or of neonatal tetany shortly after birth. The cause of the latter is this: in normal individuals, the parathyroid gland secretes parathormone, which acts upon bones, bringing about the release of calcium into the blood. In children with DiGeorge syndrome, the parathyroid gland is underdeveloped, the level of parathormone is too low to release enough calcium for normal muscle function, and the calcium shortage results in muscle spasms, or tetany.

Children who do not succumb to heart failure or tetany may die from fungal or viral infections, from which they cannot protect themselves because of the absence or underdevelopment

of the thymus. The precursors of T-lymphocytes have nowhere to mature and the entire cellular arm of immunity is thus defective. The degree of thymus underdevelopment and the resulting T-cell defects are, however, quite variable. About half the patients have a small thymic rudiment, but frequently in the wrong place. Consequently, the T-cell defect may range from absolute T-cell deficiency to almost intact T-lymphocyte compartment. Lymph nodes and spleen have striking structural deficiencies in the thymus-dependent areas. B-cell numbers and immunoglobulin levels are normal.

The nonimmunological features of the DiGeorge syndrome can be controlled by intravenous infusions of calcium (to prevent hypocalcaemic seizures) and by corrective heart surgery. Attempts have been made to correct the immunological defect by fetal thymus transplants. The results have varied to a great extent: some clinicians have reported promt improvement in the patient's immunological status, while others have seen no change in comparison to untreated patients.

Thymus development is also involved in Nezelof's syndrome, which bears the name of the French pathologist who first described it. Although present, the thymus in children with Nezelof's syndrome remains relatively undifferentiated, embryonic in character, and incapable of sustaining normal T-lymphocyte development. T-cell deficiency can be moderate to severe, B-cell defects may arise secondarily, as a result of T-cell absence. The syndrome is difficult to define and to distinquish from other disorders. It may represent a group of diseases rather than a single deficiency. The affected children fail to thrive and suffer from recurrent, predominantly viral

and fungal infections and from chronic diarrhoea. In contrast to DiGeorge syndrome, Nezelof's syndrome is inherited, but the pattern of inheritance has not been established since this disease is quite rare.

VISKOTT-ALDRICH SYNDROME

Named after the two pediatricians who described it, Viskott-Aldrich syndrome is second only to SCID in its severity as an immunodeficiency disease. Like SCID, it affects both the cellular and the humoral immune systems, but unlike SCID, it affects other systems as well. Viskott-Aldrich syndrome is characterized by a triad of symptoms - recurrent infections by all classes of microorganisms, thrombocytopenia, and eczema of the skin. The recurrent infections usually do not begin until 6 months of age. They include infections with pneumococci, meningococci, H. influenzae and other microorganisms with polysaccharide capsules, but some children also die of viral or fungal infections. Meningitis, pneumonia, sepsis, middle-ear infections, and disseminated fungal infections are a constant threat to the patients with Wiskott-Aldrich syndrome. Thrombocytopenia is already present at birth, with platelet counts ranging from 10 000 to 30 000/mm^3.

When autoimmune thrombocytopenia becomes superimposed on the normal thrombocytopenia, the counts may drop even further to a mere 1000/mm^3. Thrombocytopenia leads to severe bleeding, often in the brain, and has a fatal outcome in some 20 % of the children. Eczema is usually present by one year of age and it resembles that found in allergic children. It varies from mild eruptions in the bending surfaces of the extremities to a generalized involvement of the entire skin. The patients also have

a tendency to develop severe autoimmune disease and they show a marked increase in the frequency of malignant tumours, up to 25 % of the children develop lymphomas.

The immunological defects involve both T- and B-lymphocytes. The patients have only slightly decreased numbers of lymphocytes, but their T-cells do not respond to antigens, although they respond normally to mitogens. Particularly affected seem to be cytotoxic T-lymphocytes. The immunoglobulin levels may be normal but the proportions of the Ig classes are disturbed: IgM levels are higher, and IgE levels are exceptionally high. All the immunoglobulin classes are rapidly catabolized and their half-lives are thus considerably shortened. To maintain normal serum immunoglobulin levels, immunoglobulins are produced at rates far higher than normal. In spite of the high rate of Ig synthesis, however, the patients fail to produce specific antibodies upon immunization with an antigen and this is probably the main reason why they are so susceptible to the infection with encapsulated microorganisms such as S. pneumoniae and H. influenzae.

The nature of the defect in the Wiskott-Aldrich syndrome is not known. The disease is X-linked, and despite its pleiotropic manifestation, might be the consequence of a single primary defect, which may secondarily cause the appearence of multiple symptoms. The thrombocytopenia can abe corrected by splenectomy, suggesting that it is caused by some destructive process in the spleen. The disease can be cured by bone marrow transplantation if a histocompatible donor is available.

IMMUNODEFICIENCY WITH THYMOMA

The characteristic feature of the immunodeficiency with thymoma is the combination of hypogammaglobulinaemia with an increased incidence of thymomas. The thymomas may precede the hypogammaglobulinaemia in some patients and follow it in others. A variety of other haematological disorders accompanies this immunodeficiency disease. Over 50 % of the patients are anaemic and leucopenic, the majority of them lack eosinophils and are deficient in basophils, and about one fourth show deficient in basophils, and about one fourth show significant thrombocytopenia. Pre-B-cells and mature B-lymphocytes are absent, T-lymphocytes are present but do not seem to function properly (they suppress the activities of B-lymphocytes from healthy individuals). Consequently, both humoral and cellular immune systems are affected by the disease and the patients are sussceptible to infections in which either one or the other system is involved. Despite hypogammaglobulinaemia, autoantibodies appear frequently.

The disease afflicts adults only. It is often fatal, but this is mainly because of secondary infections. Surgical removal of the thymoma does not improve the condition and the only remedy which is successful to some extent is replacement therapy with gammaglobulin. The nature of the defect and the identity of the defective cell are not known.

ATAXIA TELANGIECTASIA

Ataxia telangiectasia is an autosomal recessive disorder characterized by a loss of the ability to coordinate muscles controlled by the cerebellum (cerebellar ataxia, from Gk a, negative, taxia, 'a drawing up in rank and file'), dilation of

capillaries in facial skin and eyeballs (oculocutaneous telangiectasia, from Gk telos, end, angeion, vessel, ektasis, a stretching out), hypersensitivity to ionizing (but not ultraviolet) radiation, lung infections, reduced number of T-cells, defective helper T-cell activity, increased incidence of certain tumours, and reduced serum levels of certain immunoglobulin classes (IgA, IgE, and IgG2). Some of the immunological defects can be explained by an underdevelopment of the thymus. The organ remains embryonic in appearence or is missing altogether. The neurological disorders are caused by a progressive neuronal degeneration, which begins at an early age, when the child begins to walk. The dilation of venous capillaries begins around the conjuctivae of the eyes and then spreads to the eyelids, ears, and neck. Other common features of the disease are premature greying of the hair, atrophy of the skin, endocrine abnormalities involving a number of organs, increased levels of serum alpha-fetoprotein, and glucose intolerance. Cells from ataxia telangiectasia patients often show chromosomal abnormalities which usually involve chromosomes 7 and 14, more specifically bands 7p14, 7q34, 14q12, and 14q32. The fact that these are two chromosomes on which immunoglobulin-encoding genes are locataed may not be accidental. The hypersensitivity to ionizing radiation, which is known to induce chromosomal breaks, also falls in line with this observation. It has been suggested that the hypersensitivity results from a defect in a DNA repair mechanism and hence the failure to restore some of the DNA strand breaks induced by the radiation.

The frequency of ataxia telangiectasia homozygotes is about 1 : 40 000 and that of heterozygotes may be as high as a 1 :

100. There may be more than one type of the disease. In genetical tests at least four complementation groups could be identified when defective genes of independent origin were brought together in the same cell. Restoration of a function by two complementing genes often suggests that the genes occupy different loci on a chromosome. Neither the genes nor the primary defects caused by them have, however, been identified.

IMMUNODEFICIENCY WITH PURINE NUCLEOSIDE PHOSPHORYLASE (PNP) DEFECT

PNP deficiency is another immune disease with a well-characterized enzyme defect - the absence of purine nucleoside phosphorylase (PNP). The enzyme catalyses the conversion of inosine to hypoxanthine (Fig. 9.2), a step following the one that involves ADA. The defect is caused by a mutation at a locus on chromosome 14, and the homozygotes for this mutation either lack the enzyme completely or produce normal levels of an inactive enzyme. The heterozygotes have half the normal enzymatic activity. PNP deficiency affects only T-lymphocytes, and perhaps only a T-cell subset, since there are reports that T-helper functions remain normal. Cytotoxic T-cells, however, are clearly affected because they cannot provide the necessary protection against viral and other infectious diseases. B-cell responses and B-cell counts appear to be normal. The disease begins between 4 months and 2 years of age.

CHRONIC MUCOCUTANEOUS CANDIDIASIS

Candida albicans, a saprophyte yeast-like organism, is a common inhabitant of the human gastrointestinal tract. Most infants are exposed to it during or shortly after birth (some 20 % of pregnant women have significant yeast vaginitis) and

approximately 5 % of infants develop oral candidiasis (thrush of the mouth) which later disappears by itself. By the end of the first year of life, almost all children have been exposed to C. albicans, as indicated by the presence of specific antibodies in their sera. In a few individuals, however, the C. albicans infection becomes chronic and may persist throughout life, with the fungus growing on the mucous membranes, skin, hair, and nails. In some patients with chronic mucocutaneous candidiasis, endocrine abnormalities may appear later in the development of the disease. They include hypoparathyroidism, Addison's disease, diabetes mellitus, and hypothyroidism. Other disorders such as ovarian insufficiency, chronic active hepatitis, pernicious anaemia, alopecia, and iron-deficiency anaemia may also become associataed with the disease. The patients are unable to respond to C. albicans antigens in vitro. Although they respond normally to all other tested antigens and they have normal numbers of T- and B-lymphocytes, and normal immunoglobulin levels. The immunological defect therefore seems to be specific for the candidal antigens and to affect only T-lymphocytes.

The severity of the disease varies from patient to patient, and several forms of the condition may actually exist. Both males and females are affected. While the spreading of the fungus can be controlled by anti-mycotic agents such as intravenously administered amphotericin B, the treatment provides only temporary relief, and as soon as the drug is withdrawn (because of toxicity at larger doses), the fungus returns within a few weeks. No corrective therapeutic treatment is availbale for the disease.

X-LINKED AGAMMAGLOBULINAEMIA

X-linked (Bruton-type) agammaglobulinaemia results from a defect in B-cell differentiation. The patients' stem cells differentiate normally up to the pre-B-cell stage, but stop there, so that no mature B-lymphocytes develop. The differentiation of T-lymphocytes proceeds normally. The patients therefore have a normal number of T-lymphocytes, normal thymus, and normal thymus-dependent areas in their lymphoid organs, but they have no plasma cells in the germinal centres of the spleen, lymph node, tonsils, and Peyer's patches. The levels of all major classes of immunoglobulins are drastically reduced, but contrary to the implication contained in the name of the disease, a basic level of immunoglobulins exists in all patients. The defect occurs in a gene or genes on the X chromosome and is recessive, which means that the immunodeficiency normally appears in boys only (some female agammaglobulinaemia patients, presumably representing rare homozygotes for the defective gene, have nevertheless been reported).

Boys who have inherited the defect usually do not show any signs of the disease until the age of 5-6 months, presumably because they are protected by passively transferred immunoglobulins from their mothers. As these immunoglobulins gradually disappear from the circulation, however, the diseasesets in the form of frequent bacterial infections. Particularly common are infections with encapsulated pathogens such as S. pneumoniae, H. influenzae, and N. meningitidis. The infections may lead to septicaemia, meningitis, chronic bronchitis, otitis media, and pneumonia, in some cases, encephalomyelitis (caused by certain strains of echovirus) also develops and leads to progressive

deterioration of the central nervous system.

The disease can be treated by periodical intramuscular inoculations of immune globulin, which establish a minimal level of immunoglobulins and provide antibody protection against at least some of the infectious diseases. The repeated intramuscular injections are painful, however, lead to local scarring, and may produce severe anaphylaxis-like reactions. An alternative is the use of intravenous plasma infusions, but each of these takes several hours, and depends on plasma from a single donor who must be hepatitis-free. Preparations of immune globulin for intravenous administration are also available, but are expensive. The infections can be controlled to a certain degree by antibiotics. In spite of all these treatments, however, patients with X-linked agammaglobulinaemia often do not survive beyond the second or third decade of their lives, respiratory failure being the principal cause of death.

COMMON VARIABLE IMMUNODEFICIENCY

Also called adult aquired agammaglobilinaemia, common variable immunodeficiency is a heterogenous group of disorders which all have hypogammaglobulinaemia in common, the disorders differ from X-linked agammaglobulinaemia in that they do not show any clear pattern of inheritance (hence the adjective "acquired"). Often even within the same family, different individuals develop different forms of the disease (hence the adjective "variable"). The disease is associated with a high incidence of infections by the same encapsulated bacteria that infect patients with X-linked agammaglobulinaemia. Some of the patients with common variable immunodeficiency, however, also show an increased incidence of fungal and protozoal infections.

They may develop autoimmune diseases (e.g. haemolytic anaemia and nondeforming arthritis), and they show an increased incidence of malignancies, especially lymphomas and reticuloendothelial neoplasms. Gastrointestinal abnormalaities, such as chronic diarrhoea, lactose intolerance, malabsorbtion, and protein-losing gastroenteropathy (disorder of the stomach or intestine), sometimes also accompany the disease.

The variability of the immunological abnormalities suggests that the disease is really a acomplex of different conditions. Some patients lack B-lymphocytes completely while others have B-lymphocytes in nearly normal nembers. In the latter group, B-lymphocytes of some patients fail to respond normally. In some patients, the defect seems to occur in the glycosylation of immunoglobulin molecules which leads to defective secretion of these molecules by plasma cells. A group of patients has also been described in which the normal B-lymphocyte response is purportedly inhibited by suppressor T-lymphocytes.

SELECTIVE IMMUNOGLOBULIN DEFICIENCIES

Several immunodeficiencies have been described in which only one class of immunoglobulins (IgA, IgM, or IgG) is affected by the defect, while all other classes function normally.

Selective IgA deficiency is a state of almost complete absence of this single immunoglobulin class. It is the most common immunodeficiency, affecting approximately one in every 800 - 3 000 persons. Although it is known to be controlled genetically, its mode of inheritance has not been elucidated. Many people with IgA deficiency are completely healthy, but others show symptoms related to the function of IgA molecules. Immunoglobulin A, as awe already know, takes part in neutralization

of infectious agents at their port of entry and in absorption of noninfectious antigens introduced into the body by breathing or with food. All five smptoms of IgA deficiency - frequent infections of paranasal sinuses and pulmonary airways, gastrointestinal problems, allergies, autoimmune reactions, and lung as well as gastrointestinal tumours - may be the consequence of a breakdown in these IgA functions. In the absence of IgA on the mucosal surfaces of the lungs, sinuses, and gastrointestinal tract, these organs are easy prey to the pathogens introduced by breathing or with food. Antigens normally bound by IgA bind instead to mast-cell IgE and cause allergic reactions. Among the accumulating antigens no longer cleared by IgA could be some that resemble the body's own molecules, so that when the body begins to mount immune reactions against these antigens, it also unleashes an attack against its own molecules. Finally, the antigen accumulating in the lungs and gastrointestinal tract is a source of constant irritation, which can eventually lead to cancer. The actual site of the defect responsible for the IgA deficiency could be either in the IgA-committed B-lymphocyte, or in a T-lymphocyte that regulates the differentiation of the IgA^+ B-cells into plasma cells.

Selective IgM deficiency, one of the rarest immunodeficiencies, is characterized by the virtual absence of IgM in the serum and normal levels of other immunoglobulin classes. Patients with IgM deficiency can mount normal antibody responses to some antigens but are unable to cope with certain bacteria, in particular pneumococci and meningococci. The patient's ability to mount T-cell reactions is unimpaired. It would be particularly intersting to know the site of the defect responsible for this

deficiency, since one is hard-pressed to explain how the synthesis of an Ig class can be suppressed without the concurrent suppression of other Ig classes when the expression of this class in the differentiation pathway precedes the expression of other immunoglobulins.

Selective deficiency of IgG subclasses affects IgG without influencing other immunoglobulin classes. The IgG class is known to consist of four subclasses and the deficiency can affect various combinations of these. The symptoms of the disease are relatively mild, consisting mainly of frequent bacterial infections, and the ailment is often diagnosed relatively late in life.

10. ACID-BASE BALANCE AND ITS DISORDERS

10.1. ACID-BASE BALANCE

The pH of the blood of normal man is alkaline, and it is maintained within the small range of about 7.37 - 7.42. A narrow range of pH is essential to normal metabolic function, probably because the activities of protein macromolecules depend on an optimal pH. By definition, pH is the negative logarithm of the hydrogen ion concentration in a solution. In man, a pH range of 6.5 to 7.8 (corresponding to a hydrogen ion concentration, $[H^+]$, from 250 nmol/l to 18 nmol/l) covers the range of values which appear to be compatible with life (Tab. 10.1).

Tab. 10.1. Important values of pH and H+ ion concentration

	$[H^+]$ nmmol/l	pH
Normal values in extracellular fluid	44 - 36	7.36 - 7.44
ACIDOSIS	> 44	< 7.36
Acidosis at maximal physical exercise	94	7.10
Maximal acidosis compatible with life	158	6.80
ALKALOSIS	< 36	> 7.44
Maximal alkalosis compatible with life	20	7.70

In recent years a number of experts have preferred a notation of hydrogen ion concentration, rather than pH in analyzing acid-base disturbances. Both expressions have advantages and disadvantages. One of the major arguments for the use of the hydrogen ion concentration was, that because the pH scale is logarithmic, an equal change in pH may reflect unequal changes in H^+ concentration. Thus a change in pH from 7.0 to 7.1 represents a change in $[H^+]$ from about 100 to 79 nanomoles per liter (nmol/l), whereas a change in pH from 7.3 to 7.4 represents only half as great a change in $[H^+]$, namely, from 50 to 40 nmol/l. However, for the shake of clarity, we shall use only pH in this chapter.

10.2. THE PROBLEM OF HUMAN ACID-BASE BALANCE

Despite its essential alkalinity, the mammalian body normally produces large amounts of acid from two major sources:

1. Some 13 000 to 20 000 mmoles of CO_2 is produced daily as the result of oxidative metabolism; when hydrated, this yields the weak acid, carbonic acid

$$CO_2 + H_2O \ast H_2CO_3 \ast H^+ + HCO_3^- \qquad \text{(Equation 1)}$$

Because H_2CO_3 is in equilibrium with a volatile component, CO_2, it is often referred to as *volatile acid*.

2. In most developed countries, where meat constitutes a large part of the diet, there is also a net daily production of some 40 to 60 mmol of noncarbonic acids, mainly from the catabolism of proteins, and to a lesser extent of phospholipids. Because these acids, unlike H_2CO_3, are not in equilibrium with a volatile component, they are known as *nonvolatile*, or *fixed*,

acids (in vegetarians, or in others whose diet consists mainly of vegetables and fruits, the net production of noncarbonic constituents consists of alkalis).

In certain physiological and pathological states, the production of nonvolatile acids may rise tenfold. Examples include the production of lactic acid during muscular exercise and states of hypoxia, and the production of aceto-acetic acid and betaoxybutiric acid during uncontrolled diabetes mellitus.

DEFENSE MECHANISMS

Thus, the problem of mammalian H^+ balance is the defense of normal alkalinity in the face of constant acid load. Under these conditions, acid base balance is preserved by coordinated actions of three lines of defense:

First line defense - fast physicochemical buffering. A buffer is a mixture of either a weak acid and its conjugate base, or of a weak base and its conjugate acid (acid is here defined as a H^+ donor and base as a H^+ receptor). The buffers of physiological importance in mammals are all of the first type. They have been listed in Tab. 10.3. These buffer systems are by no means limited to the plasma. They are found in all phases of body fluids - plasma, interstitial fluid, intracellular fluid and bone. The bicarbonate system predominates in plasma and interstitial fluid, while organic phosphates and proteins (especially hemoglobin) predominate in the intracellular spaces.

Hydrogen ions generated or lost in the activation of Equation 1 are interchangeable with H^+ in any other buffer system in that particular body fluid compartment, and this *isohydric principle* means that a description of the acid-base status ba-

Tab. 10.2. Physiological values of pH and $[H^+]$ in various cells and body fluids

	$[H^+]$	pH
Intracellular fluid		
Red blood cells	53 nmol/l	7.28
Platelets	100 nmol/l	7.00
Muscle cells	126 nmol/l	6.90
Osteoblasts	3 nmol/l	8.50
Prostatic cells	32 umol/l	4.50
Other body fluids and excretory products		
Ventricular fluid	63 - 1 nmol/l	1.2 - 3.0
Duodenal fluid	316 - 25 nmol/l	6.5 - 7.6
Large-bowel fluid	13 - 10 nmol/l	7.9 - 8.0
Bile	631 - 3 nmol/l	6.2 - 8.5
Urine	32000 - 10 nmol/l	4.5 - 8.0

Tab. 10.3. Important buffer systems of the mammalial body fluid compartments. The locations refer to qantitative predominance and are not exlusive, except for hemoglobin in erythrocytes.

PROTOTYPE	BICARBONATE	PROTEINS	ORGANIC PHOSPHATES
HBuf	H_2CO_3	Hn Prot	Hn Phos
Buf-	HCO_3^-	Prot-	Phos n-

		Hemoglobin	Other Proteins
EXTRACELLULAR		INTRACELLULAR	INTRACELLULAR
Plasma	Red blood cells		Other cells
Intestinal fluid			

sed on the bicarbonate buffer system will also describe the H^+ changes resulting from all other buffer systems in that compartment. It is important to note here, that intracellular $[H^+]$ is very different from that in the extracellular fluid and varies

a little from cell to cell (Tab. 10.2). Human red blood cells have an intracellular pH of around 7.2 when blood pH is 7.4 and pCO_2 5.3 kPa. Human muscle cells (the most abundant tissue in the body) when resting, have an intracellular pH of 6.8-7.0, but as lactic acid is produced by muscular contraction, human muscle intracellular pH falls towards 6.4. In clinical practice, however, the acid-base status of the body is best assessed by examination of arterial blood, where measurements of pH, pCO_2 and pO_2 are part of everyday clinical practice.

The following reaction is the prototype for physicochemical buffering:

strong acid + buffer salt * neutral salt + week acid. Insofar, as physicochemical buffering reduces sthe amount of buffer salt and increases the amount of weak acid, this type of reaction only minimizes, but by no means prevents, a decrease in pH. This point can be illustrated by simple calculations utilizing the **Henderson-Hasselbach equation**, as it applies to the bicarbonate system:

$$pH = pK' + \log \frac{[HCO_3^-]}{[\text{dissolved } CO_2] + [H_2CO_3]} \qquad \text{(Equation 2)}$$

For plasma at 38 degrees of Celsius the pK' (negataive logarithm of the apparent dissociation constant) is 6.1. The concentration of CO_2 in plasma is proportional to the partial pressure of CO_2 (pCO_2) in plasma, which is relatively easy to determine. Thus, ignoring the trace amounts that exists as H_2CO_3, this equation can be rewritten in a form that is most useful in physiological and clinical practice:

$$pH = 6.1 + \log \frac{[HCO_3^-]}{[pCO_3]} \qquad \text{(Equation 3)}$$

It a larger amount of stronger acid were added to the extracellular fluid, physicochemical buffering would decrease the numerator and increase the denominator. If this were to occur in a "closed system", i.e. without a ventilatory system that can eliminate the newly generated CO_2, the pH would drop, sometimes to fatal level. This dire consequence is prevented by the second line of defense, which, like physicochemical buffering, comes into play within seconds or minutes after the acid load.

Second line defense - fast respiratory component. Actually, all of the H_2CO_3 that was produced through physicochemical buffering is converted to CO_2 and H_2O (Equation 1), and the CO_2 is excreted by the lungs. Respiratory compensation goes further, however. As a result of the lower pH of the blood, alveolar ventilation is increased (hyperventilation), so that alveolar and hence arterial pCO_2 is decreased. Consequently the pH is returned toward, but not quite to, the normal value.

Third line defense - slow renal component Although respiratory compensation has, within minutes, restored the pH almost to normal, the stores of the main extracellular buffer have been seriously depleted. This fact is reflected in the decrease of the concentration of the $[HCO_3^-]$. Furthermore, some excessive H^+still remains within the body as weak acid. Both of these remaining abnormalities are corrected by the kidneys, which excrete H^+ and simultaneously replenish the depleted HCO_3^- stores. This process is much slower one than fhe first two lines

of defense, requiring hours to days rather than seconds or minutes to come into play.

10.3. CONCEPT OF METABOLIC AND RESPIRATORY DISTURBANCES

Primary disturbances. Inspection of one form of the Henderson-Hasselbach equation (Equation 3) makes clear that an abnormality of plasma pH can result from a primary deviation of either the nominator, $[HCO_3^-]$, or of the denumerator, $[pCO_2]$. Since the concentration of the latter is regulated by the rate of alveolar ventilation, any disturbance in H^+ balance that results from a primary change in pCO_2 is called **respiratory**. Thus, hyperventilation and retention of CO_2 lead to a reduction in pH that is called respiratory acidosis, hyperventilation and a fall in pCO_2 lead to a rise in pH that is called respiratory alkalosis. Changes in the concentration of HCO_3^- are brought about most commonly by the net addition or net loss of nonvolatile (fixed) acids or bases, which are derived mainly from metabolic processes. Hence, any abnormality of pH resulting primarily from a change in HCO_3^- is called **metabolic**. For example, the endogenous production of aceto-acetic acid and beta-hydroxybutyric acid in uncontrolled diabetes mellitus leads to metabolic acidosis, while prolonged vomiting with loss of gastric HCl results in metabolic alkalosis.

Thus, there are four primary disturbances of H^+ balance (more often called acid-base balance): a) respiratory acidosis, b) respiratory alkalosis, c) metabolic acidosis and d) metabolic alkalosis.

Compensatory responses. Most primary disturbances in H^+ ba-

lance tend to elicit a secondary response that partially corrects the pH. In the example mentioned earlier in this chapter, the addition of a strong acid led to a decrease of [HCO_3^-] and hence to metabolic acidosis. This disturbance was largerly compensated for by the second line of defense, in which alveolar hyperventilation lowered the [pCO_2] and thereby adjusted the pH to a near normal value. This example illustrates two important points: (a) that a compensatory response involves the system opposite to the one that caused the primary disturbance (e.g. metabolic acidosis is compensated for by a respiratory response, and vice versa) and (b) that compensation shifts the pH toward but not to the normal value. The reason for this is probably that the compensatory hyperventilation is controlled not only by the pH but also by the [pCO_2]. Since primary disturbances may be either metabolic or respiratory in origin, and may cause either acidosis or alkalosis, there are four general types of compensatory responses: 1) metabolic acidosis is compensated for within minutes by alveolar hyperventilation, 2) metabolic alkalosis may be accompanied by a respiratory compensation that decreases alveolar ventilation, but this response is not invariable, 3) if the primary disturbance is respiratory acidosis, it is usually compensated for by increased renal excretion of H^+ and increased renal reabsorption of HCO_3^-, this response usually takes days to develop, 4) finally, respiratory alkalosis is compensated for by a change in the metabolic component of Equation 3, namely, by decreased renal excretion of H+ and decreased renal reabsorption of HCO_3^-.

Mixed disturbances. Sometimes two primary disturbances, usually one respiratory and the other metabolic, occur simulta-

neously in the same individual. Such patients are said to have "mixed" acid-base disturbances. For example, a patient who manifests alveolar hyperventilation from emphysema of the lung may also have an obstructed duodenal ulcer leading to loss of HCl though vomiting. This patient would have a mixed disturbance of respiratory acidosis and metabolic alkalosis. Another patient may have both emphysema with retention of CO_2, and renal failure, which leads to the retention of fixed acids. This patient would have a mixed disturbance of respiratory acidosis and metabolic alkalosis. There are four major mixed disturbances: a) respiratory acidosis plus metabolic acidosis, b) respiratory acidosis plus respiratory alkalosis, c) respiratory alkalosis plus metabolic acidosis and d) respiratory alkalosis plus metabolic alkalosis.

10.4. ACID-BASE VALUES USED IN CLINICAL PRACTICE

Ideally, disorders of H^+ balance are analyzed by determining the **pH** and pCO_2 of an arterial blood sample, which is obtained anaerobically. In routine clinical practice, arterial blood is usually replaced by "arterialized" capillary blood sample collected into a heparinized capillary. Ideally, the sample should be analysed immediately. This is because lood is continuiously metabolizing to decrease oxygen tension and to increase pCO_2 and decrease pH.

In most instances, these two values, pH and pCO_2, are then plotted on some diagram or nomogram of the Henderson-Hasselbach equation (Equation 3), so that the value of the third variable in Equation 3, **actual bicarbonate**

$[HCO_3^-]$, can be read off or estimated (Fig. 10.1). The non-respiratory (i.e. metabolic) component may be described in various terms, including **base excess**, which is defined as the amount of base required to titrate a blood sample to pH of 7.4 and arterial pCO_2 of 5.3 kPa at 38°C. This term base excess has now replaced the older term **standard bicarbonate** which was defined as the $[HCO_3^-]$ of whole blood when this had been equilibrated with pCO_2 of 5.3 kPa at 38°C. (Tab. 10.4). The complete analysis usually includes a determination of arterial pO_2 and **hematocrit** (Hct) values, because these variables influence the buffering characteristics of blood and provide further information about the respiratory system, which plays such an important role, primarily or secondarily, in H^+ balance.

Tab. 10.4. Reference values of acid-base parameters used in clinical practice

Parameter	Definition	Reference values
$[H^+]$	Hydrogen ion concentration	
pH	Negative logarithm of $[H^+]$	
pCO_2	Partial pressure of carbonic dioxyde	
Actual bicarbonate	Actual bicarbonate concentration (usually assessed by a nomogram)	
Standard bicarbonate	The $[HCO_2^-]$ of whole blood when this had been equilibrated with pCO_2 of 5.3 kPa at 38°C.	
Base excess	The amount of base required to titrate a blood sample to pH of 7.4 and arterial pCO_2 of 5.3 kPa at 38°C	
pO_2	Partial pressure of oxygen	

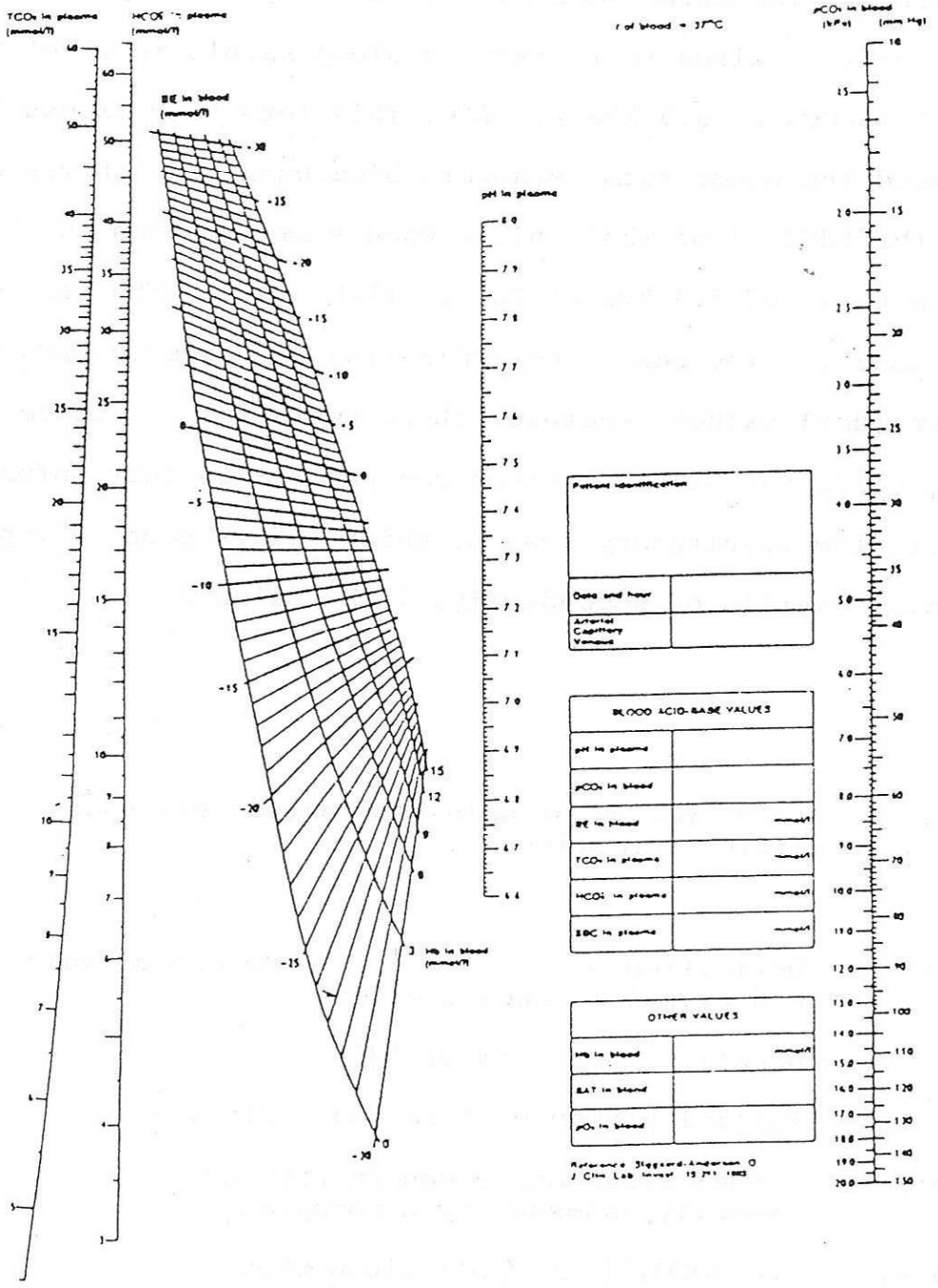

Fig. 10.1. Siggaard-Andersen acid-base nomogram. To use of this nomogram align the values of pCO_2 and pH and read the value of base excess (or base deficit) from the line intersecting the appropriate value of the hemoglobin concentration. Note that hemoglobin concentration is given in mmol/l (1 mmol = 16.1 g). Calculated total CO_2 and plasma HCO_3^- also can be determined by extrapolation the line.

10.5. METHODS OF EVALUATION

The currently used interpolation method for evaluating acid-base balance was introduced by Astrup and a more complex nomogram, still based on the Henderson-Hasselbach equation, to assess acid-base balance was described by Siggaard-Andersen (1962). It depends on the equilibration of an arterial blood sample with gases of two known values of pCO_2 and measurement of the pH of these blood samples after equilibration. When values of pCO_2 and pH in arterial blood are determined, a line is drawn between these values on the nomogram and continued to intersect a line corresponding to the measured hemoglobin concentration in arterial blood. This intersection corresponds to the calculated base excess (BE). Many blood gas analyzers have programmed into them the parameters and equations based on the Siggaard-Andersen nomogram and thus provide values of base excess.

It has to be mentioned at this point, that an automatic use of the diagrams without understanding the dynamics of H+ balance and sufficient knowledge about the medical history and physical examination ot the patient can lead to misinterpretations and incorrect therapy. This information can promote the correct diagnosis in the great majority of patients and, in many instances, laboratory tests should serve mainly to confirm, qualify and quantify diagnostic impressions based on the medical history and physical examination. With this remark in mind, we can introduce two additional useful tools that may be of help for correct diagnosis - namely, the calculation of the anion gap and the use of the 95 % confidence bands.

Calculation of the anion gap is based on the principle of

electroneutrality of the plasma, in other words on an equal concentrations of negatively charged anionic and positively charged cationic particles in this body compartment. Fig. 10.2 shows that most of the electrical charges on the major cation of plasma, namely Na^+, are neutralized by Cl^- and HCO_3^-. The remaining charges on Na^+, which amount 10-16 mmol/l, are covered by some of the negative charges on other anions in plasma, namely phosphate, sulfate, organic acids and proteins. The sum of these charges is called anion "gap", because they cover the gap of positive charges on Na^+ left, so to speak, by Cl^- and HCO_3^-. The anion gap is thus defined as the difference between $[Na^+]$ and the sum of $[Cl^-]$ and $[HCO_3^-]$, all expressed in mmol/l. The gap is sometimes also referred to as the "sum of unmeasured anions", pointing to the fact, that the anions neutralizing the remaining charges on Na^+ are not routinely measured in experimental or clinical conditions. Because this gap is typically increased or unchanged in certain metabolic acidotic states (Tab. 10.5), its quick calculation at the bedside is often of tremendous help in analyzing such disturbances. The gap increases during metabolic acidotic states which are characterised by production of excess organic or inorganic anions (e.g. in lactic acidosis or in renal failure). If these anions are not readily reabsorbable from the glomerular filtrate, homeostatic alkalinisation will be facilitated due to enhanced H^+ secretion in exchange for Na^+.

The use of the confidence bands (Fig. 10.3) help to distinguish chronic from acute respiratory disorders and to predict how much compensatory change in $[HCO_3^-]$ may be expected in pure respiratory disturbances or how much compensatory change in

Tab. 10.5. Causes of metabolic acidosis. The disorders have been divided into those that are usually accompanied by an increased anion gap and those in which the gap is usually normal. This division reflects the major clinical usefulness of the anion gap.

Increased anion gap
- Uncontrolled diabetes insipidus
- Renal failure
- Lactic acidosis
- Administration, ingestion or intoxication
  - ethyl alcohol with starvation and production of keto-acids
  - salicylate, methyl alcohol, paraldehyde, ethylene glycol

Normal anion gap
- Diarrhea or loss of other gastrointestinal fluids with high $[HCO_3^-]$ through fistulas or surgical dainage
- Renal tubular acidosis
- Administration, ingestion of intoxication
  - NH_4Cl, carbonic anhydrase inhibitors

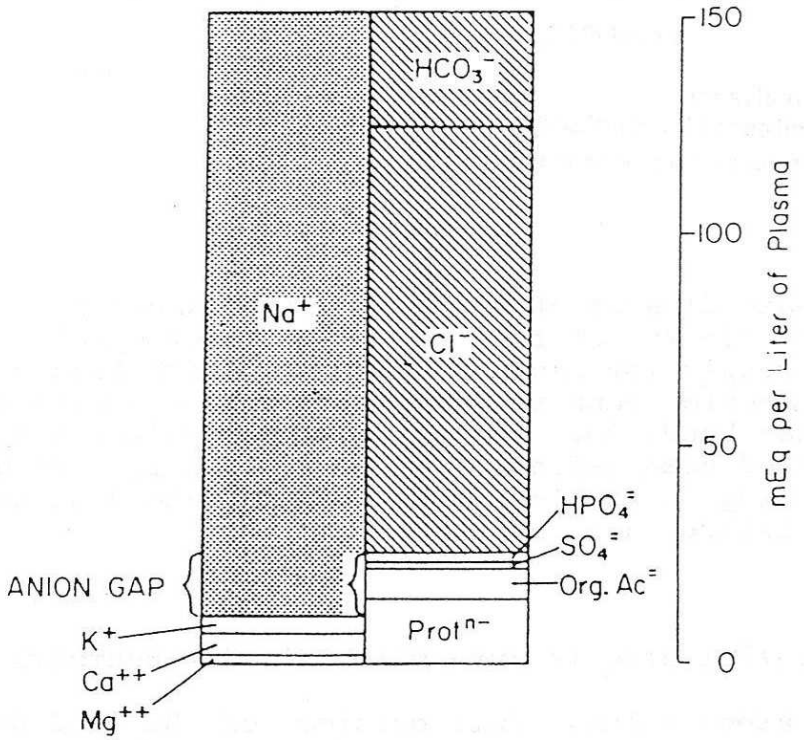

Fig. 10.2. Anion gap for normal plasma.

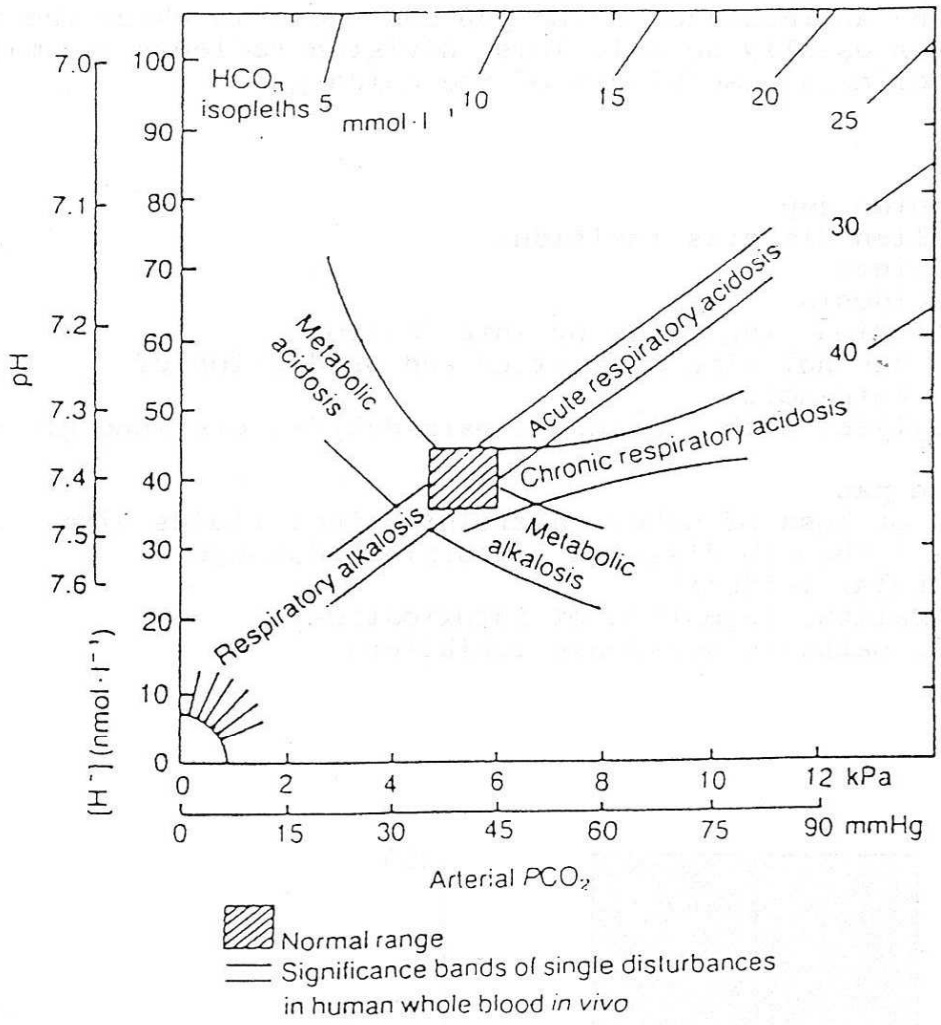

Fig. 10.3. Acid-base diagram of arterial blood showing the 95 percent confidence limits of the $[H^-]/pCO_2$ relationship for acute and chronic respiratory acidosis in vivo, with linear extrapolation of the acute band to include respiratory alkalosis and the significance bands for in vivo metabolic alkalosis and acidosis. The shaded area defines the normal range. The HCO_3 isopleths, originating in the left bottom corner and continuing to the top of the figure, are also shown.

$[pCO_2]$ should be anticipated in pure metabolic derangements. If a patient's laboratory values fall outside of the confidence bands, a mixed disturbance of H^+ balance is indicated. If however, the values fall within the bands, this fact will not necessarily reflect an uncomplicated primary disturbance.

RESPIRATORY ACID-BASE DISTURBANCES

Here the major problem is either retention of CO_2 with a resultant raised pCO_2, or over-breathing, for whatever cause, resulting in low arterial pCO_2.

Respiratory acidosis

If normal healthy subjects are given CO_2 to breathe, and their arterial blood is sampled when the inspired CO_2 is held constant over periods of several minutes, the relationship between pCO_2 and pH in their arterial blood is defined by the band marked "*acute respiratory acidosis*" on the Fig. 10.3. If however, such CO_2 inhalation is continued for several days, an experiment which has never been carried out in man but has been studied in dogs, then the renal tubules re-absorb HCO_3^-, increasing the $[HCO_3^-]$ in the plasma and increasing the buffering power of the blood, so that the pH falls although the pCO_2 remains high. The pH/pCO_2 relationships in arterial blood now lie within the "*chronic respiratory acidosis*" band on Fig. 10.3. Many studies of patients with chronic CO_2 retention from lung sidease (e.g. chronic bronchitis and emphysema, cystic fibrosis, etc.), who were not receiving diuretics or other drugs that may affect acid-base balance, show pH/pCO_2 relationships which lie within this significance band for chronic respiratory acidosis.

Respiratory alkalosis

In contrast, when the primary disturbance is hyperventilation, arterial pCO_2 is reduced indicating *respiratory alkalosis*. This occurs in patients with acute pneumonia, pulmonary edema, bronchial asthma, etc. and almost certainly arises from stimulation of vagal afferent fibers within the lungs, because

vagal blockade can prevent hyperventilation. It used to think, that the hyperventilation in these conditions is due to concomitant hypoxia which arises, but clinical experience shows that administering oxygen to these patients can correct he arterial hypoxemia but does not restore the pCO_2 to normal. Thus, the hypoxemia seen in patients with pneumonia, pulmonary edema, bronchial asthma etc. contributes very little to he excessive ventilation of these patients. However, in high altitude, hypoxia is indeed the primary stimulus to he hyperventilation which reduces the pCO_2. In these circumstances, as in the clinical conditions discussed above, the patient suffers from respiratory alkalosis, with pH/pCO_2 relationships lying within the significance band of "respiratory alkalosis" shown on Fig. 10.3.

The figure reveals also an important teaching point. I is obvious that one could draw an isopleth of $[HCO_3^-]$ radiating from the origin at a level between 25 and 30 mmol/l (i.e. rather within the normal range) which could encompass the whole range of values of pCO_2 from the most severe respiratory alkalosis to the most severe acute respiratory acidosis. This clearly shows, that measurement of the $[HCO_3^-]$ alone is not an adequate description of acid-base balance in respiratory disturbances.

METABOLIC ACID-BASE DISTURBANCES

The primary abnormality in metabolic acid-base disturbances is either a change in $[H^+]$ or a change in $[HCO_3^-]$.

Metabolic acidosis

Metabolic acidosis causes an increase in ventilation, stimulated by the rise in arterial $[H^+]$ (decrease in arterial pH) acting on the central chemoreceptors mediated through the ce-

rebrospinal fluid and via the peripheral carotid and aortic chemoreceptors. The 95 % confidence limits for metabolic acidosis shown in Fig. 10.3 were derived from patients with untreated diabetic ketoacidosis, chronic stable renal failure, repeated hemodialysis for chronic renal failure and untreated cholera. In clinical practice the severity of metabolic acidosis can often be assessed by $[HCO_3^-]$ values alone, usually by measuring this value in venous blood. Fig. 10.2 shows, that this is possible because the significance bands of arterial pH/pCO_2 values cross the HCO_3^- isopleths at almost right angles.

Assessment of base excess is of particular interest in treating patients with metabolic acidosis. Base excess (BE) given in mmol/l, in fact is the amount of bicarbonate (in mmoles) which would be necessary to add to every liter of plasma (or, rather extracellular fluid) to restore normal acid-base balance. Assuming that bicarbonate is distributed mainly in the extracellular compartment roughly corresponding to about 1/3 of body weight, the amount of intravenous bicarbonate to be administered can be calculated by the formula: BE x 0.3 x body weight. It should be emphasized, however, that in clinical practice it is not possible to prescribe a safe and affective treatment of a given degree of acid-base disturbance from one measurement alone, but that sequential measurements of arterial H^+/pCO_2 relationships are essential during the management of such conditions in order to restore normal function. Thus, the practice of calculating the amount of intravenous bicarbonate by the above mentioned formula in expectation that this will definitely correct any metabolic component of an acid-base disbalance, in fact, fraught with hazard. It is far more useful in

practice to administer, say, one half or one quarter of this calculated dose and titrate the patient individually towards correction of his acid-base disturbance.

Metabolic alkalosis

Accepted causes of metabolic alkalosis include loss of gastric juice due to high upper intestinal obstruction or nasogastric suction (e.g. pyloric stenosis) and, of course, administration of large doses of either absorbable alkali orally, or intravenous bicarbonate. In these conditions the urinary chloride concentration is usually low, there is also a less common group of disorders causing metabolic alkalosis in which the urinary chloride level is high. These disorders include hyperaldosteronism, Bartter's syndrome (hyperplasia of the granular cells of the juxtaglomerular apparatus of the kidney with associated renal potassium wasting, normotension and hyporesponsiveness of arterial blood pressure to infused angiotensin II), Coushing's syndrome, ingestion of licorice and most commonly, severe and prolonged potassium deficiency. In profound metabolic alkalosis there is usually severe reduction in the volume of the extracellular fluid, so that renal function is impaired, which often compounds the potassium depletion.

Although it seems reasonable to expect pCO_2 to rise as arterial pH increases, since this would be expected to remove a drive to breathing, in clinical practice the rise of pCO_2 is rarely significant. An arterial pH above 7.52 rarely arises in practice, and the width of this curvilinear significance band indicates that even in such causes the CO_2 will still usually be normal. However, severe metabolic alkalosis may threaten life, interacting with potassium deficiency and hypokalemia to

affect cardiac function by generating arrythmias. If alkalosis persists despite a normal extracellular fluid volume and normal potassium concentration, then acetazolamide (a potent inhibitor of carbonic anhydrase, producing metabolic acidosis) may be necessary. If this fails, direct infusion of hydrochloric acid may be indicated.

11. INFLAMMATION

Inflammation is the series of responses which occurs in a living tissue when it is injured. The biologic significance of inflammation is that of a defensive and protective mechanism. The process of inflammation is the succession of interdependent reactions by which defensive factors, such as phagocytes, complement, and immunoglobulins can give access into the area of tissue injury or microbial invasion and this process also includes repair of injury by the proliferation of fibroblasts, endothelial cells, and collagen production.

Celsus described the cardinal signs of inflammation: rubor (redness), owing to the overloading of all vessels of the affected tissue; calor (heat), because a more than normal amount of heat is supplied to such tissue by an increase in the blood supply; tumor (swelling), because of the great increase of fluids and cell exudation into the inflamed tissue; dolor (pain), owing to the pressure of nerve endings by the overfilled vessels, exudation, and the effect of vasoactive factors. A fifth cardinal sign, functio laesa (loss of function) was added later and is secondary to pain and swelling of the tissue; it is less a symptom than a resulting condition.

Only a local inflammatory response occurs in the majority of injuries such as localized skin inflammation or rheumatoid arthritis. In the case of virulent infection or major trauma the generalized effects of inflammation consistently occurs. These include fever, neutrophilia, lethargy, and eventually muscle wasting.

CLASSIFICATION OF INFLAMMATION

Inflammation is classified according to its duration as **acute**, **subacute** or **chronic**. Acute inflammation has very different characteristics from chronic inflammation. The distinction is sometimes not very sharp since features characteristic of one group may be found in combination with features of the other group in varying proportions. The main response to injury in acute inflammation is the accumulation of fluids and cells, mainly neutrophils, later macrophages, in injured area. The fluids and cells together are referred to as exudate. When the irritant is not degradable by the phagocytes (inorganic or organic material such as silica particles or suture threads, the presence of certain bacteria such as Mycobacterium tuberculosis), the irritant persists and leads to chronic inflammation. There is the continual arrival of new macrophages and fibroblasts and deposition of collagen around the irritant focus. This type of lesion is called a granuloma.

Inflammation is termed acute if it lasts a matter of hours or days; chronic if it lasts weeks, months, or years. Acute inflammation may terminate, or may decrease and become subacute, or may persist and become chronic. Certain injurous agents induce a cellular reaction of the chronic type, without a significant acute phase, e.g., silica or the tubercle bacillus. The main features of this type of inflammation are the presence of fibrous connective tissue often with small blood vessels (granuloma) and the predominance of mononuclear cells.

Inflammatory mechanisms are divided into two broad groups (1) **immunological** and (2) **non-immunological**. Inflammatory changes immunologically mediated include hypersensitivity reactions

(e.g., anaphylactic reactions, immune complex reaction of the Arthrus type, and cell- mediated or delayed hypersensitivity reactions). These immunologically mediated tissue injuries can produce adverse reactions ranging from minor local tissue irritation to selective destruction of organs or even sudden death. Non - immunological mechanisms also include some immunological components, such as complement and immunoglobulins.

INJUROUS AGENTS

Inflammation could be initiated by a variety of injurous agents. The list of living and non-living agents that have the capacity to injure living tissue is unlimited. These agents have been grouped as follows: mechanical, such as trauma; chemical, such as corrosive acids, alkalis, and also bile and urine when they escape into the tissues; microorganisms, such as bacteria, viruses, fungi, and protozoa, as well as larger parasitic organisms; irradiation, including heat, cold, and ultraviolet light; necrotic tissue, such as infarcts; and immunological, due to antigen - antibody reaction.

Local damage of the living tissue in infectious injury may be produced by cell-bound or secreted toxins that are cytotoxic or interfere with normal function of a particaular tissue. Trauma results in direct tissue damage by mechanical contact. Irradiation concentrates an increase in energy to cells or parts of cells. Metaboloic processes and components of the cell may be altered by the increase in energy.

The inflammatory response is essential for the survival of the organism by defending against all forms of injury and allowing the healing process. When this response is diminished by cytotoxic drugs or by immunosuppression, low infectious micro-

organisms may cause serious problems. On the other hand, the inflammatory response may be inappropriate and directly harmful to the organism as it is in the immune response called hypersensitivity and in some other lesions.

11.1. ACUTE INFLAMMATION

Acute inflammation develops within a few hours after tissue injury. This process is the succession of changes which occurs in a living tissue when it is injured.

Local inflammatory response can be divided into four stages which proceed in a regular sequence.

(1) Injury to local or migrating cells; trauma.

(2) The production of mediators of inflammation and increased vascular permeability.

(3) Exudation of intravascular fluids, polymorphonuclear and mononuclear phagocytes, their emigration and accumulation.

(4) Cellular proliferation and repair.

INJURY TO CELLS

An injurous agent causes injury to local or migrating cells. Trauma, such as crushing and cutting injuries, and also thermal, and electrical injuries and irradiation result in direct tissue damage. Organism prevents entrance of microorganisms into the inner tissues of the body by physical barriers and secretions (e.g.,gastric and nasal). If the bacteria enter into the tissue, the organism differentiates self from nonself and uses the initial lines of defence against substances recognized as nonself. The organism confines the infection, prevents their spreading, and eliminates the microorganisms locally.

MEDIATORS OF INFLAMMATION AND INCREASED PERMEABILITY

Following specific or non-specific recognition of nonself substances, amplification systems are activated and lead to the production of local mediators of inflammation. These induce the tissue responses responsible for the inflammatory signs. An inflammatory mediator is any endogenous substance whose levels increase at the site of the inflammatory trauma in association with the appearance at least one tissue response or structural change. These biologically active substances participate in defence reactions, namely in inflammation and hypersensitivity. Inflammation is basically a beneficial defence reaction, whereas hypersensitivity is a harmful response.

Mediators are released from cells upon stimulation from cellular granules or are produced continuously or are derived from plasma sources. The action of the mediator can be either short-term, acting for a relatively short period of time or long-term, lasting for many hours. Inflammatory mediators are classified according to their mode of action:a) the direct acting, i.e., histamine, bradykinin, prostaglandins, which act via pharmacological receptors; b) lytic enzymes of cell or plasma origin which cause direct damage to the tissue or cells; and c) chemotactic factors which stimulate cell migration to the site of inflammation. A complex interplay of activation and suppression mechanisms modulates the type and magnitude of inflammatory response. The difference in this response brought about by different traumatic stimuli depends on the relative proportions of the different types of mediators released as well as on the duration of their release.The main biological activities associated with mediators of inflammation are increase

in vascular permeability and regional blood flow. The magnitude of fluid exudation depends on mediators acting concomitantly on vascular permeability and vasomotor tonus. The edema is the result of the simultaneous action of several mediators, the concentration of which may vary during the development of the process. The other activities are contraction and relaxation of smooth muscles and the ability to attract cells to a particular spot in the tissue (chemotaxis).

Mediators of Inflammation

Biologically active amines, histamine and serotonin (5-hydroxytryptamine, 5 HT).

Histamine is produced in tissue mast cells and circulating basophils and it is stored in metachromatic granules. When these cells are exposed to the physical injury, antigenic challenge of IgE (immunoglobulin E)- sensitized cells or C3a and C5a (complement cleavage products) and certain chemicals such as bee venom, histamine is released and exerts its biological effects via specific receptors on the surface of the target cells. This agent has diverse biologic activities. Histamine affects the blood vessels, smooth muscles, and exocrine glands. In general, it constricts large blood vessels and arterioles, whereas it dilates minute vessels, capillaries and venules. Vasodilatation is accompanied by an increase in vascular permeability. Histamine increases also permeability in larger vessels by causing the cells of the endothelial lining to separate. Histamine causes smooth muscle contraction in the bronchi, bronchioli, uterus, bladder, and gastrointestinal tract and causes direct stimulation of exocrine glands of the stomach. The other activities of histamine include depression of leuko-

cyte chemotaxis, blockage of T - lymphocyte function, and depression of further histamine release from mast cells and basophils. Histamine thus may modulate both acute and chronic inflammatory response.

Serotonin is present in CNS as one of the neurotransmitters. Most mammals have serotonin in the platelet granules, including humans, which have serotonin also in the intestinal mucosa. It acts on smooth muscles and nerves. It also causes vasoconstriction, stimulates collagen formation and causes leakage of venules.

Lipid mediators. When a cell is injured or stimulated, phospholipids, the main constituents of the plasma membrane, are cleaved off and fatty acids are released, among them arachidonic acid, the most important of these fatty acids. Metabolism of arachidonic acid is initiated either by the cyclo - oxygenase or by 5 - lipoxygenase. Under the influence of cyclo-oxygenase arachidonic acid is converted to prostaglandins (PG: PGE_2, $PGF_2\alpha$), thromboxanes (TXA_2), and prostacycline (PGI_2). Under the influence of 5 - lipoxigenase or 15 - lipoxygenase it is converted to leukotrienes or lipoxines (LX). Prostaglandins, prostacyclin, thromboxanes, leukotrienes, and lipoxins are formed in bursts in response to stimulus such as injurous trauma. Cells do not store these substances but have the capacity to generate them when their membranes are injured. Their action is restricted to the area around the sites of synthesis. Some of these substances and/or their effects are not involved in inflammation.

Prostaglandins display a wide range of biological effects, including inflammatory process. Prostaglandin E_2 (PGE_2) is for-

med in renal collection tube cells, gastric mucosa, platelets, and leukocytes. An important source of PGE_2 in immunologic reaction is the macrophage. PGE_2 modulates a number of inflammatory events. PGE_2 is effective in producing vasodilatation, sensitizes the pain receptors to stimulation and causes hyperalgesia, i.e., a state in which overt pain can be aroused by normally painless mechanical stimulation, act synergically with other inflammatory mediators to potentiate the increase in vascular permeability (edema) and overt pain. It is also pyrogenic and promotes platelet aggregation. Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) contracts bronchial and uterine smooth muscle and is a vasoconstrictor in some uterine vessels.

Thromboxane A_2 (TxA_2) is formed almost exclusively by platelets. TxA_2 is a vasoconstrictor and constricts vascular and bronchial smooth muscle and also stimulates aggregation of platelets.

Prostacycline (PGI_2) is formed by vascular endothelium and subendothelium and is a vasodilator. PGI_2 dilates both bronchial and vascular smooth muscle. It also inhibits the aggregation and adherence of platelets and adherence of neutrophils to damaged epithelium. It might serve both to limit deposition of platelets and leukocytes following vascular injury.

Leukotrienes (LT) are syntheized by granulocytes, monocytes, mast cells, and macrophages. LTB_4 is involved in inflammation as a potent chemotactic factor for neutrophils. It stimulates adhesion, migration, aggregation, enzyme release, and generation of superoxide by polymorphonuclear leukocytes. A mixture of LTC_4, LTD_4, and LTE_4 is identical with slow reacting substance of anaphylaxis.

Lipoxins are formed by granulocytes, monocytes, mast cells, and macrophages. They cause dilatation of microvasculature, chemotaxis, and contraction of smooth muscle.

Complement cleavage products. The comlement system plays a key role in promoting the elimination of microorganisms from the blood stream and it functions as the generation of the acute inflammatory response in tissues. Two complement cleavage products, C3a and C5a, are mediators of inflammation. C3a and C5a mediate a number of biologic responses, including vasodilatation, increased capillary permeability, and smooth muscle contraction. C5a is also a very potent chemotactic factor for polymorphonuclear leukocytes, monocytes, and macrophages. It is the factor that causes degranulation of mast cells and basophils.

Kinin system. Kinins are small basic peptides present in plasma alpha - 2 - globulins in an inactive form as kininogens. Kininogens are activated by plasma proteases called kallikreins. Kallikrein is a plasma enzyme system present in and released from neutrophils, and it generates the formation of kinins. The most important of kinins is bradykinin. Kinins increase vascular permeability, cause vasodilatation, and smooth muscle contraction.They also stimulate pain receptors and induce pain.

The platelet - activating factor (PAF) is a potent mediator. PAF is normally absent in the membranes of unstimulated cells but it is synthesized and released upon stimulation from platelets, granulocytes, monocytes, macrophages, mast cells, lymphocytes, and endothelial cells. The stimuli leading to synthesis of PAF are immunological, e.g.,antigen, IL-1, IgE antibodies and nonimmunological, e.g.,chemotactic factors, throm-

bin, phagocytic stimuli. PAF causes platelet aggregation and degranulation, releasing substances such as serotonin and thromboxane A_2. PAF is potent neutrophil activator and chemoattractant, it causes neutrophil aggregation and increased cell adherence, chemotaxis, enhanced respiratory burst, superoxide production, and the production of arachidonic acid metabolites. It also increases vascular permeability and induces constriction of smooth muscles in the gut and the lungs.

Interleukin - 1 (IL-1) is one out of the group of intercellular messengers, called lymphokines.Lymphokines are secreted by isolated cells of the immune system and they are not produced by unstimulated cells. Lymphokines promote activities aimed at th elimination of invading parasites and the repair of damaged tissue. IL-1 is produced by macrophages, microglial cells, mesangeal cells, monocytes, neutrophils, fibroblasts, keratinocytes, endothelial cells, astrocytes, Langerhans cells, and natural killer cells after stimulation. The stimulus can be, e.g., bacterial lipopolysaccharide, solid particles, immune complexes, cell injury, and activated T lymphocytes. The biologic activties of IL-1 are diverse and they may be grouped as follows: the effect on growth and differentiation, tissue catabolism, the effects on the central nervous system, and inflammation. Changes in inflammation process induced by IL-1 include:stimulation of bone marrow and the release of polymorphonuclear neutrophls (PMNs) into the circulation and their margination; it acts as a chemotactic factor on PMNs and causes their emigration into the tissue in large numbers where it stimulates their oxidative metabolism; it activates neutrophils and eosinophils to release lysosomal enzymes; IL-1 acts as the chemo-

tactic factor on macrophages, stimula- tes them to produce prostaglandins and enhances their cyto- cidal (tumoricidal) activity. It also acts as a pyrogen.

Lysosomal enzymes are contained in subcellular organelles of neutrophils and other cells, termed lysosomal granules or lysosomes. Lysosomal enzymes digest antigens following phagocytosis. Lysosomal granules also contain antimicrobial constituents such as myeloperoxidase and lactoferrin. The components of the lysosome may be released into the surrounding tissue and cause destruction of extracellular tissue structures seen in a number of inflammatory responses, e.g., various forms of arthrtis, the Arthus reaction, and nephrotoxic nephritis.The components of the lysosome are released into the surrounding tissue after cell death or disruption of the cell membrane, the escape of the lysosomal enzymes during phagocytosis, especially if the foreign substance is too large or during the formation of secondary lysosomes. Neutral proteinases from lysosomes, such as collagenase, elastase, cathepsin G, and SH - dependent proteinases are capable of destroying extracellular structures. The neutral proteinases of polymorphonuclear leukocytes and macrophages are also involved in the generation of chemotactic activity through their direct action on complement components such as C5, in the formation of vasoactive substances by releasing kinin substances from plasma kininogen as well as in participating in blood coagulation and fibrinolysis.

Events in the microcirculation

The smallest vessels play a dominant role in acute inflammation. The appearance of local inflammatory mediators induces vasodilataion of the local small blood vessels, the increase of

blood flow, the increase of vascular permeability, and the accumulation of fluid in the tissue.

There may be a transient constriction of arterioles around the mild injury such as mild thermal burns. Immediately after injury there is usually a vasodilatation of arterioles and venules and the precapillary sphincters open. The blood flow through the injured area increases rapidly and the pressures in all vessels rise considerably. Mediators of inflammation increase vascular permeability and there is an immediate opening of the endothelial intercellular junctions of postcapillary venules. At this stage there is a cosiderable loss of blood fluid and protein to the tissues. Initially, the blood flow is very rapid and cells become packed more tightly into the central part of the flowing blood. As the vessels become so leaky, the viscosity of the blood increases and there is considerable resistance to the flow of blood. This is followed by a gradual decrease of the blood flow through still dilated vessels. The red cells aggregate together and the palisading of leukocytes and platelets in the venules become great. The blood flow may finally cease entirely in some vessels and they form distended columns of tightly packed cells. Endothelial injury may activate components of the coagulation system and induce formation of microthrombosis. Nutrition may be so reduced that parts of the tissue become necrotic. Stasis is caused by the increase in vascular permeability, plasma exudes into the tissues, where it causes edema. In many instances the flow gradually begins again and eventually returns to normal.

As the rate of flow decreases, leukocytes move into the peripheral (plasmatic) zone of the stream (the outer stream in

contact with the vascular endothelium) and adhere to the endothelium (margination), and subsequently emigrate into the surrounding tissues.

Increased vascular permeability in the early stages of local acute inflammation causes swelling (edema) which is brought about only by the accumulation of fluid in the tissues. This increased permeability has a biphasic character. The initial phase begins immediately after injury and lasts about 1 h. The injurous agent itself can cause damage the vessels and make them leak but this first increase is mainly due to a release of histamie. A delayed second phase occurs several hours after the onset of inflammation. Kinins may be the mediators of the delayed permeability respose acting concomitantly with other mediators on vascular permeability. The endothelial and perivascular cells of the blood vessels contract so that they are pulled apart and gaps appear. Some of these gaps become plugged by platelets, but others remain open and plasma pours out. These cells in the nonmuscular venules contain microfilaments (contractile material).

Wherever there are gaps in the endothelium, plasma escapes, the protein concentration in the interstitial fluid rises and the result is an exudate. When the exudate is in the tissues, there is activation of the complement system, the coagulation system, and the fibrinolytic system. The cleavage fragments of the complement system include factors causing vasodilatation and increased vascular permeability (C3a, C5a), chemotactic factor (C5a), opsonins (C3b,C4b), a factor that causes PMNs degranulation and oxidative activa- tion (C5a), and the membrane attack complex (C5b6789) that lyses the plasma membranes of

gram - negative bacteria.

Hageman factor (factor XII) of the coagulation system is activated by injury of vascular endothelium, e.g., by bacterial lipopolysaccharides and it triggers the cloting cascade with formation of thrombin. Thrombin acts on the fibrinogen in tissue exudate to produce insoluble fibrin which acts as a barrier to prevent spreading of infection and to eliminate the microorganisms locally. Activated Hageman factor also triggers the fibrinolytic system by activating plasminogen activator, which converts plasminogen to plasmin. Plasmin digests fibrinogen and fibrin (fibrinolysis) and cleaves complement to give chemotactic cleavage products for neutrophils. Activated Hageman factor finally as a prekallikrein activator activates prekallikrein to form kallikrein. Kallikrein then cleaves kininogen (plasma alpha 2 - globulins) to produce kinins.The most important is bradykinin.

EMIGRATION AND ACCUMULATION OF POLYMORPHONUCLEAR AND MONONUCLEAR PHAGOCYTES

The leukocytes are separated into two major groups: the phagocytes and immunocytes (lymphocytes and plasma cells). The phagocytes can be divided into the polymorphonuclear cells or granulocytes and the monocyte - macrophages (mononuclear phagocytes). The monocytes belong to the leukocytes circulating in the blood while the macrophages are encountered in the connective and lymphoid tissue as free and fixed macrophages. The granulocytes and the monocyte - macrophages are bone marrow derived.

Polymorphonuclear phagocytes

Mature polymorphonuclear leukocytes are divided into neu-

trophils, eosinophils, and basophils.

Neutrophils, also called polymorphonuclear neutrophils (PMN) are the most common leukocytes and account for about two - third of white cells in the blood. The half - life of the mature neutrophil in the blood is only 6 -8 h. The major function of neutrophils is the ingestion and destruction of foreign material through phagocytosis. They form the first line of defence against foreign intruders. Neutrophils have a well developed capacity for locomotion, are able to respond to a very large number of chemotactic factors, are highly phagocytic, they contain in the azurophilic granules (or lysosomes) acid hydrolases, lysozyme, myeloperoxidase, neutral proteases, cationic proteins with bactericidal activity and NADPH - oxidase; in the secondary or specific granules alkaline phosphatase, lysozyme, lactoferrin, and collagenase; they kill microorganisms by combined destructive action of oxidative and nonoxidative processes. Neutrophils accumulate at the sites of acute inflammation. In the early stages of the acute inflammatory response, the predominant cells infiltrating the tissues are the polymorphonuclear neutrophils (PMNs). In the tissue infected with pyogenic bacteria, the influx of neutrophils is greatly enhanced and sustained. As the intensity of the acute inflammatory response subsides, the prevailing cells are the mononuclear phagocytes or macrophages. More neutrophils emigrate into the inflammatory focus in the early stages, whereas more macrophages enter the focus later.

Eosinophils share the same properties with neutro- phils. They respond to the same chemotactic stimuli as neutrophils. They have some capacity for phagocytosis, but they are less ef-

ficient than neutrophils. The small eosinophilic granules contain acid phosphatase, glucuronidase, cathepsin, ribonuclease, arylsulphatase, and other enzymes. The large granules contain major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil - derived neurotoxin. Eosinophils play a particular role in hypersensitivity and helmintic infestations. Helmints can be destroyed by eosinophil degranulation, i.e., by the release of granule contents onto the worm cuticle, and kill it by the respiratory burst and potent lytic enzymes. This may be the major function of these cells. Accumulation of eosinophils at the sites of immediate hypersensitivity (type I hypersensitivity) is an important feature; they possess the ability to cause tissue damage.

Basophils are the least numerous and have little phagocytic capability, their granules contain histamine and serotonin. Their importance lies in participation in immediate hypersensitivity. They possess the membrane receptors for the Fc portion of IgE and two basophil - bound IgE interact with specific antigen causing basophil degranulation and immediate hypersensitivity.

Mononuclear Phagocytes

Mononuclear phagocytes (monocyte - macrophages) comprise a variety of phagocytic cells, related by origin and function, which include the blood monocytes and macrophages in various tissues.

Monocytes are cells circulating in the blood and later enter the tissue to develop into macrophages.

Macrophages are localized in connective tissue and lymphatic organs as fixed and free macrophages. The mature macropha-

ges live for weeks to months.

Monocytes and macrophages costitute the **mononuclear phagocyte system.**

Depending on the localization of macrophages the fixed varieties are called Kupffer cells (liver), microglial cells (CNS), osteoclasts (bone), mesangial cells (kidney), macrophages of spleen and lymph nodes, and histiocytes (connective tissue); free macrophages are macrophages of serosal cavities (pleural, peritoneal, and pericardial), and alveolar macrophages. Macrophages have the capacity to fuse together to form the giant cells and to turn into epitheloid cells.

Mononuclear phagocytes eliminate deleterious material by phagocytosis (particles larger than 1 μm) or pinocytosis (particles smaller than 1 μm). They ingest worn out cells, cell debris, antigen - antibody complexes, invading pathogens and neoplastic cells. Ingestion increases by many folds if the microorganisms are coated with antibody or complement (opsonization).This occurs because the macrophages have the Fc receptors and the C3 receptors. Like neutrophils, mononuclear phagocytes are able to kill many bacteria following ingestion. When macrophages enter into the inflamed tissue,they are stimulated by inflammatory stimuli and develop increased phagocytic ability such as enhanced phagocytic activity, increased levels of lysosomal enzymes, and increased neutral protease secretion. The respiratory burst is much less intense than in neutrophils. Obligate intracellular pathogens such as M. tuberculosis are only killed with some delay or not at all. For the destruction of these pathogens, macrophage activation is essential. The activation of macrophages requires an interaction of the macrophage

with a stimulus such as bacterial products and with the T cell lymphokine. Activated macrophages can deal with such pathogens much more efficiently, by virtue of the enhanced ingestion and intracellular killing. These pathogens are able to infect and multiply within unactivated macrophages. Mononuclear phagocytes are mobile and exhibit chemotaxis. The particulate material is brought to the fixed macrophages by the blood stream.

Macrophages produce and secrete a great variety of biologically important substances such as interleukin - 1 (pyrogen), lysozyme, complement components, and leukotriene. Activated macrophages produce also neutral proteases such as collagenase and elastase, playing role in the healing, plasminogen activator, and reactive oxygen. Some of these substances are released only during the phagocytic process.

Macrophages play an important role in several aspects of immune response, not only in degrading complex structures (microorganisms) and processing the resulting antigens but also in cell - mediated (delayed) hypersensitivity, in presenting these antigens to T lymphocytes, which are stimulated to produce biologically active substances (e.g.,lymphokines) and, in turn, activation of macrophages by the lymphokine produced by the T cells.

Inflammatory cell accumulation

The accumulation of polymorphonuclear phagocytes and tissue macrophages at the sites of tissue injury or antigen plays a central role in eradicating organisms in tissues and mucosal surfaces. Polymorphonuclear neutrophils (PMNs) are concerned principally with the destruction of extracellular pathogens, whereas mononuclear phagocytes control the microorganisms which

are able to survive intracellularly and against which neutrophils are ineffective. PMNs and free macrophages are motile, respond to chemotactic stimuli, have surface receptors for C5a and LTB_4 (leukotriene B_4), and move directionally along the concentration gradient of the chemoatractant. Both engulf particles, degranulate the lysosomal contituents, and produce reactive oxygen, though microbicidal activity is much lower in unactivated macrophages than in neutrophils.

In response to tissue injury or infection, large numbers of **neutrophils** are mobilized to the site within a short time. They are attracted to the site of injury by chemotactic substances. All polymorphonuclar cells exit the circulation by diapedesis and emigration. They must first adhere to vascular endothelial cells to migrate into the tissue. The increase in adherence is the result in an increase in the amount of specific surface glycoproteins on the PMN surface. There is an increase in the amount of these glycoproteins on the surface after exposure to inflammatory mediators C5a, LTB_4, and TNF - α (tumor necrosis factor). After adherence to the endotheliun neutrophils migrate into tissues. Neutrophils extend pseudopodia and penetrate the endothelium at the cell junction (**diapedesis**). Once in the tissues the movement of PMN is directed along the concetration gradient of the chemotactic substances from the injured site (**chemotaxis**). The phenomenon chemotaxis concerns the purposeful directional movement of cells towards a specific chemical stimulus. Neutrophils are able to respond to a very large number of chemotactic factors, but the most important is complement cleavage product C5a. Activation of arachidonic acid metabolism leads to the generation of leukotriene B_4 (LTB_4), which is

another strong chemoatractant. C5a is released by proteases from neutrophils in the inflammatory area and also released during the activation of the complement pathway either directly by bacteria or by immune complexes. LTB_4 is also produced by stimulated neutrophils. The production of C5a and LTB_4 by neutrophils at the site of inflammation reinforces generation of chemotactic factors that attract more neutrophils to the inflammed region.Also N - formylated oligopeptides, intermediates in bacterial protein synthesis, are important chemotactic factors, released from bacteria that have been damaged or killed. Emigration of large numbers of PMNs is accompanied by an increased production of these cells in the bone marrow. PMNs move more rapidly in the tissues than monocyte - macrophages. Macrophages are attracted to the site of inflammation not only to bacterial products, C5a, some cytokines, and other substances but also to damaged neutrophils in the site of invasion. Dying neutrophils release elastase and collagenase and so generate macrophage chemotactic factors. During an acute inflammatory response the majority of the macrophages recruited to the site of the lesion are newly - formed cells.

Phagocytosis

Once the neutrophils have come to the site of invasion, the cells began to ingest viable microbes with subsequent destruction of the pathogens. This process consists of several stages: opsonization and adherence of phagocytes to bacteria, ingestion and digestion.

Opsonization. In order to initiate phagocytosis, the stimulating foreign particle must first bc bound to the membrane of phagocyte. Adherence does not happen spontaneously and not all

bacteria are equally susceptible to phagocytosis. Phagocytes and foreign particles have a nega- tive charge and repel each other, so it must be neutralized by coating with a positively charged protein. Substances that coat foreign particles and facilitate phagocytosis are called opsonins. There are two general classes of opsonins: (a) **IgG** antibodies and (b) complement cleavage fragment **C3b**. Phagocytes have Fc receptors specific for the Fc region of IgG antibodies, and complement receptors specific for the C3b fragment. Antibody binds to antigenic sites on a foreign particle, the Fc portion of IgG is directed outward. Phagocytes adhere to Fc portion of immunoglobulins. It is in the absence of serum. After depositing the complement fragment C3b on foreign particles for which phagocytes have receptors, they bind to the foreign particles. This process is called **opsonization**. Phagocytes can ingest bacteria that do not have a capsule without the mediation of opsonins. The surfaces of encapsulated bacteria on the other hand, repel approaching phagocytes. Such bacteria are phagocytosed only when opsonized. Some microorganisms can activate the alternative pathway of complement in the absence of antibodies and the generated C3 fragments exert an opsonizing effects almost immediately after infection.

Ingestion. Immediately following the attachement of the foreign particle to the phagocyte, the particle is engulfed. The phagocyte extends the pseudopodia over and around it, completely covers the particle and this particle is drawn into the cell, so the particle finds itself in a vesicle, the **phagosome**. The ingestion advances more rapidly, if the particle is hydrophobic and a coating by C3b or IgG increases hydrophobicity.

Digestion. Intracellular granules and lysosomes migrate to the phagocytic vesicle, fuse with it, and empty their contents into it, thus forming a phagocytic lysosome or **phagolysosome**. This emptying of granules into the phagocytic vacuole is called degranulation. It may begin while vacuole is still open to the cell exterior and this extracellular degranulation may be responsible for the tissue injury during some immunological reactions. Extracellular release of enzymes in lysosomes may also occur when the phagocytes die and these enzymes may damage the affected tissues, e.g., in rheumatoid arthritis.

Bactericidal activity (killing) involves the combined action of oxidative (the respiratoty burst) and nonoxidative processes.

Respiratory burst

The resoiratory burst is characterized by an increased consumption of oxygen. The increase is more pronounced for the neutrophils than for the other phagocytes. Neutrophils produce from this oxygen superoxide anion ($\cdot O_2^-$), H_2O_2, and possibly hydroxyl radical ($OH\cdot$), and singlet oxygen ($^1O_2$). The burst is activated by the contact with opsonized or unopsonized bacteria, antigen - antibody complexes, chemo- tactic factors (C5a, LTB_4), indigestible particles, and other stimuli. The enzyme NADPH - oxidase on the cell surface is activated that converts NADPH to $NADP^+$ with the release of electrons. The molecule of oxygen accepts two donated electrons resulting in the generation of superoxide anion ($\cdot O_2^-$). Most of $\cdot O_2^-$ produced reacts with itself to generate $\mathbf{H_2O_2}$, a well- known germicidal agent. Superoxide anion does not accumulate and does not participate in the attack but H_2O_2 is stable. Neutrophil myeloperoxidase

catalyzes the oxidative reactions, but the most important are that between hydrogen peroxide and intracellular halide ions (Cl^-, Br^-, I^-, or SCN^-). This neutrophil myeloperoxidase catalyses the reaction of H_2O_2 with Cl^- to produce the **hypochloride anion** (OCl^-). It is a very powerful oxidant that rapidly attacks many biological molecules and enhances the bactericidal activity of the lysosomal enzymes. Hypochloride anion can react with neutrophils and yield oxidant with the properties of a chloramine. Myeloperoxidase can be released intracellularly into the phagolysosome, as well as extracellulary. Macrophages lose their granular peroxidase during maturation and therefore mature macrophages are not very good at killing ingested microorganisms. The respiratory burst generates two oxygen species, i.e., hydroxyl radical ($OH^\bullet$) and singlet oxygen ($^1O_2$) and they are able to attack and kill ingested microorganisms but evidence for the direct participation of these active oxygen species in microbicidal reactions is scarce.

Oxygen independent bactericidy

The enzymes of granules and lysosomes released into the phagolysosome may destroy bacterial wall and kill some microorganisms. Lysozyme is a cationic protein and can destroy the cell walls of gram - positive bacteria. The neutral and basic proteases, e.g., elastase, cathepsin G and multiple hydrolytic enzymes of the neutrophiles are potentially capable of damaging cells and tissues. Lactoferrin withholds iron required by some bacteria. There is also a decrease in pH within the phagolysosome which has a direct antimicrobicidal effects.

After digestion of microbes the vacuoles are disintegrated and their contents are released into the cytoplasm; after death

of neutrophils they are released into the surrounding tissues where they are responsible for the tissue damage of a numer of hypersensitivity reactions, e.g., Arthus reaction.

Activated Macrophages

Many bacteria are rapidly killed following ingestion by macrophages, but others remain and multiply in the phagocytic vacuoles, even if bound to antibody and complement cleavage product before engulfing. These are the intracellular pathogens such as bacteria Mycobacterium tuberculosis, Listeria monocytogenes, Salmonella typhosa, Brucella abortus, Chlamidia, Rikettsia; fungi Candida albicans, Histoplasma capsulatum; protozoa Toxoplasma gondii, Malarial plasmodia, Trypanosoma cruzi, and Leishmania donovani. These intracellular pathogens are killed or inhibited in their growth when the macrophage becomes activated by cellular immune reaction.

Activated macrophages are macrophages, which by their increased capacity to kill pathogens, could enhance the host resistance. The action of the activated macrophages is non - specific. The macrophages will inactivate bacteria regardless of the organism that produced the infection. Here is an example: An animal recovering from an infection with L. monocytogenes shows increased resistance to infection by M. tuberculosis.

The activation of macrophages requires an interaction of the macrophage with one of a number of stimuli such as bacterial products and with the T cell lymphokines. This is the control that the activated macrophages cause inflammation only at the site of bacterial infection.

The general mechanism is thought to be that after phagocytosis the antigen molecules are processed by partial catabolism

and antigen fragments transported to the surface membrane and processed immunogenic substances are presented to neighboring T lymphocytes. T lymphocytes with receptors for the presented antigenic determinants are stimulated. Stimulated T lymphocytes release lymphokines that activate macrophages.

The most important feature of the activated macrophages is their enhanced microbicidal activity which is poorly developed in unactivated macrophages. Intracellular killing of pathogens by activated magrophages results mainly from production of reactive oxygen metabolites such as hydrogen peroxide and/or superoxide anion. Proteases, such as collagenase and elastase, released from the activated macrophages could possess a cytocidal activity. Activated macrophages have the ability to kill pathogens too large for ingestion. They spill extracellularly the contents of their lysosomes on the particles and release hydrogen peroxide or inhibit mitochondrial respiration by the enzyme arginine deiminase synthesized by the activated macrophages.

Activated macrophages occur at a certain stage of the infection and their occurrence coincides with the appearance of delayed (type IV) hypersensitivity. The activation of macrophages is therefore a cellular hypersensitivity phenomenon. This activation declines with the decline in amounts of persisting viable pathogens in the body. In some cases, however, even the activated macrophages are not capable of destroying the invasive pathogens. If the macrophages and T cells fail to get rid of the intracellular pathogens, the host then begins the process of blocking off the infested area, and a granulomatous tissue (granuloma), characteristic of chronic inflammation, develops.

This immunologic mechanism gives rise to the formation of granulomas in certain chronic infectious diseases such as tuberculosis. In this case macrohages accumulate around the persistent pathogens and look like squamous epithelium - thus the name epitheloid cells. Multinucleated giant cells result from fusion of the cytoplasms of the macrophages. Epitheloid and giant cells are the feature of tubercles.

CELLULAR PROLIFERATION AND REPAIR

Repair begins soon after injury occurs. The mechanisms of repair are built right into the inflammatory reaction. Almost any injury involves the proliferation of **fibroblasts** which have very great capablities to proliferate and **endothelial cells** in injured tissue. Two main agents of repair are the **macrophage**, which cleans up the site and the **fibroblast**, which patches up the damage. They work always at the periphery of an inflammatory focus.

Injury induces an acute inflammatory reaction with accumulation of neutrophils. Several hours later the macrophages begin to arrive. They remove not only microorganisms but also fibrin, dead polymorphs, damaged tissues, and other cellular material. They release collagenases and elastases destroying connective tissue, stimulate the production of plasmin dissolving fibrinogen and fibrin, and release interleukin (IL - 1) promoting the proliferation of fibroblasts and stimulating synthesis of the collagen by fibroblasts required to repair any tissue damage. The macrophages also release angiogenic factors, as the response on local ischemia, stimulating the growth of the new capillaries. The aim of repair is to return normal functional and morphological integrity to the tissue. Prolifera-

tion and repair on one side and other inflammatory processes on the other side often occur coincidently, except in chronic inflammation without an acute phase where there are two main features: the mononuclear predominance and granulomatous tissue.

Complete repair without forming scar tissue. It means restitution to normality, and it can occur only where injury to the tissues has been slight. An example may be surgical knife cut or lobar pneumonia. In the absence of significant bacterial contamination the inflammatory exudate is rapidly removed. The fibrin, red cells, and dead polymorphs are phagocytosed and digested by macrophages. There is only some contribution by fibroblast to repair. Repair is complete if the exudate is removed quickly and completely.

Complete repair with scar tissue occurs in major injuries where full functional integrity is not likely to be restored. It means the process by which exudate, dead tissue, blood clot or thrombus is replaced by a mass of new connective tissue, known as granulation tissue, and ultimately by a fibrous mass called a scar. In this case macro- phages gradually remove debris and exudate from inflammatory focus and the space is replaced by new capillaries and by proliferated fibroblasts which actively produce structural material such as collagen fibers and a few elastic fibers forming the granulation tissue. Then there is the gradual disappearance of cellular nuclei and blood vessels in this cellular tissue and the collagen fibers compact and contract; the final result is a dense fibrous scar.

Regeneration implies that lost cells are replaced by cells of the same kind. Some specific tissue cells regenerate well, e.g., connective tissue, skin, epithelium, smooth muscle, and

liver. Therefore, depending upon the tissue involved regeneration of cells may also take place.

11.2. CHRONIC INFLAMMATION

Inflammation is called chronic if it lasts weeks, months, or years. It can arise from acute and can arise as chronic without any acute phase.

Chronic inflammation arises from acute if the causative agent, capable of inducing acute inflammation, persists and the inflammatory reaction also persists. Pyogenic bacteria cause acute inflammation with purulent exudate and prolonged exudation. If this exudate is not completely removed, an effort is made to confine this process, and a wall of proliferated fibroblasts and collagen fibers is gradually formed around the inflammatory focus, now called an abscess. Neutrophils cross into the cavity of the abscess via capillaries in the connective tissue. The abscess is a localized collection of pus in a cavity formed by the disintegration of tissues. The abscess may remain active for prolonged period.

Chronic inflammation without any acute phase develops if the offending material or organisms cannot be destroyed by the macrophages or neutrophils and the chronic cellular reaction persists without a significant acute phase. Such inflammatory lesion is produced by organisms, which survive inside phagocytic cells or they are parasitic eggs, causing granulomatous disease such as tuberculosis, leprosy, salmonelosis, listeriosis, syphilis, Q - fever, histoplasmosis, hypersensitivity pneumonitis, schisostoiasis, trichiniasis, filariasis. Such lesion is also produced by vegetable or mineral fibers not digested by

phagocytes such as silica, talk, asbestos crystals, fats and oils, sterile foreign bodies. These antigens or foreign materials induce the prolonged and continuous chemotactic stimulation and continual inflow of new macrophages and fibroblasts and accumulation of collagen fibers around the focus. This specifically organized structure is called **granuloma**. Granuloma is a locally confined, persistent response to the presence of a generally poorly soluble substance, mediated through the accumulation, proliferation, and differentiation of cells of the mononuclear phagocyte system. Grauloma is, thus, an aggregate of inflammatory cells, usually arranged concentrically around the causative agent.

Granulomatous inflammation is classified as nonimmunologic (nonantigenic) and immunologic (antigenic). **Persistent inactive nonimmunologic irritant** (e.g. talk) causes rapid development of the lesion and quick diminution, leaving a rim of cells walling off the foreign body. **Persistent active nonimmunologic irritant** (e.g. silica) is toxic substance and has the lethal effect on macrophages with their consequent rapid turnover. In the case of silicosis, there is perpetuation of the lesion because silica particles that are released as the macrophages die are soon taken up by other macrophages. Macrophage enzymes are released and macrophages also release IL - 1, stimulating production and deposition of collagen by fibroblasts. This leads to excessive tissue damage and dense local fibrosis. **Persistent immunologic irritant** causes the inflammatory response which correlates a cell mediated or delayed (type IV) hypersensitivity. Persistent immunologic stimulation, the release of lymphokines and IL - 1 by macrophages form a shape of a classic granuloma which

contains the agent in the center surrounded by a concentring ring of diffuse mixture of macrophages (macrophages called epitheloid and multinucleated giant cells) surrounded first by lymphocytes around the outside of the main mass of epitheloid cells and then connective tissue where fibroblasts progressively lay down collagen. This tissue contains numbers of new blood vessels. Fibrous tissue (fibrosis) is formed in this zone. In this manner, undigestible microorganisms such as the tubercle bacillus can be walled off from the rest of the body even though living bacilli are inside the granuloma.

Intracellular pathogens, phagocytozed by macrophages and surviving inside cells, interfere with the normal killing unles macrophages are not activated. An example of such interference may be Mycobacterium tuberculosis which produces sulfatides that inhibit fusion of phagosomes and lysosomes, or outer surface of Mycobacterium lepraemurium which contains waxes, not readily digested by lysosomal enzymes. The host then blocks off the infected area, and a granulomatous tissue develops.

12. HYPOXIA

12.1. DEFINITION AND CLASSIFICATION OF HYPOXIA

Hypoxia is defined as **lack of oxygen in tissues** (oxygen starvation) which can arise because of various reasons. Hypoxia is usually preceded by **hypoxemia** - decreased concentration of oxygen in blood. However, there can exist also hypoxia without hypoxemia. **Hypercapnia** (elevated concentration of CO_2 in blood) is frequently found in hypoxic state, but not in every case. Sometimes hypoxia even can be accomapnied by **hypocapnia** (Tabs. 12.1 and 12.2)

The consequences of hypoxia depend on

* time in which the hypoxia develops;
* duration of hypoxia;
* intensity and form of hypoxia itself;
* sensitivity of the affected tissues.

In order to understand the pathogenetic mechanism leading to hypoxia, one should first clarify the basic external and internal factors responsible for adequate oxygen supply of the body. These are as follows:

1/ sufficient amount of oxygen in the inhaled air;

2/ proper exchange of gases in the lungs (ventilation, diffusion and perfusion);

3/ sufficient amount of hemoglobim capable of oxygen transfer;

4/ proper function of cardiovascular system;

5/ ability of tissues to use oxygen (terminal oxidation).

The cardiovascular system **[4]** integrates the various parts of the system: it assures that **sufficient amount of**

oxygem [1] through basic fucntions of the respiratory system [2] and the red blood cells [3] may reach the tissues [5].

The peculiar structure and function of hemoglobin enables to pick up oxygen very quickly in the lungs, transport it at a considerable concentration to the tissues and - last but not least - release it through a very sophisticated molecular regulatory mechanism in the capillaries of the body.

Tab. 12.1. Concentration of gases in the air and in the blood

Gas	AIR		BLOOD	
	atmosphere	alveolus	arteries	venes
	Partial pressure in kPa and (torr)			
O_2	21 (158)	13.5 (101)	12.5 (95)	5.3 (40)
CO_2	0.04 (0.3)	5.3 (40)	5.3 (40)	6.1 (46)
N_2	79.6 (597)	76.2 (572)	76.2 (572)	76.2 (572)
H_2O*	0.7 (5)	6.3 (47)	6.3 (47)	6.3 (47)

*vapor pressure

Tab. 12.2. Concentration of O_2 and CO_2 in the blood

BLOOD		ARTERIAL	VENOUS
O_2 bound to Hb	ml/l	210	154
	mmol/l	9.2	6.8
O_2 physically dissolved	ml/l	2.9	12.0
	mmol/l	0.13	0.53
Ratio bound/dissolved		72	23
Hb saturation with O_2 (SaO_2)	%	97.1	75.0
CO_2*	ml/l	596	638
	mmol/l	22.1	24.4

*Detailed data on CO_2 and $^-HCO_3$ in plasma and red cells -> acidobasic balance, Chapter 10

The classification of various forms of hypoxia corresponds with the above mentioned external and internal factors leading to hypoxia:

* **Hypoxic hypoxia** (disturbed condition [1] or [2]). The basic reason is decreased concentration or pressure of oxygen in the inhaled air or a disturbance of respiration.

* **Anemic hypoxia** (disturbed condition [3]. Various anemic states, pathologic forms or blockage of hemoglobin transport function.

* **Circulatory (ischemic) hypoxia** (disturbed condition [4]). Total or local disturbance of circulation, venous stagnation of blood or ischemisation due to arterial closure.

* **Histotoxic hypoxia** (disturbed function [5]). Inability to utilize oxygen in the tissues.

Another possibilty is the classification of hypoxia according to its time course:

In everyday medical practice the most frequent hypoxia is that one which is an accompanying feature of chronic diseases of respiratory and cardiovascular system or blood and therefore this is **chronic hypoxia.**

Acute hypoxia is a less frequent condition. In addition to the acute form of **mountain sickness** it occurs at **suffocation, airway obstruction, inhibition of the respiratory centre, acute heart failure and shock.**

A special form from the view of onset speed is the **fulminant** hypoxia. It occurs for example at the damage of pressure cabin of airplanes in altitudes above 10 km.In this case the oxygen pressure in external environment is lower than the pressure in venous blood and therefore the organism **delivers** oxygen onto the external environment. Unconsciousness arises

very soon and after 1-2 minutes death appears as a consequence of respiratory centre failure, without cramps, without any warning signs.

12.2. HYPOXIC HYPOXIA

The most common form of hypoxia, characterized by reduced oxygen tension in pulmonary capillaries. Hypoxic hypoxia can be observed at low oxygen pressure in inhaled air, at decreased ventilation of lungs or in the case of extensive pathological processes in respiratory system leading to alveolo-capillary blockage. At reduced oxygen tension in pulmonary capillaries hemoglobin in red cells cannot fully saturate with oxygen and therefore hypoxemia develops.

The actual reasons of hypoxic hypoxia can ve divided to two groups:

1/ **"There is nothing to breathe"** - in high altitude above the sea level (normal concetration of oxygen, decreaed pressure) or if the air contains decreased proprotion of oxygen despite its normal pressure (e.g. in cellars filled with CO_2 instead of oxygen. Attention do not confound with CO intoxication!).

2/ **"There is nothing to breathe with"** - disturbed basic functions of the respiratory system outlined in Tab. 12.3 and described in detail in Special Pathological Physiology - Chapter Respiration.

If compensatory hyperventilation is possible and effective (e.g. in mountain climbing) **hypocapnia and respiratory alkalosis** can ensue. Alkalosis further deteriorates the release of oxygen from hemoglobin. On the contrary, if the respiration is insufficient, **hypercapnia and respiratory acidosis** can develop.

Tab. 12.3. The most frequent respiratory diseases leading to hypoxic hypoxia

HYPOVENTILATION
*Obstruction of airways (corpus alienum, tumor) *Paralysis of respiratory muscles (poliomyelitis) *Deformities of skeleton (kyphoscoliosis) *Disturbances or inhibition of respiratory centre (morphin, respiratory distress syndrome, hypocapnia) *Superficial breathing (pain, injuries)
BLOCKADE OF ALVEOLOCAPILLARY DIFFUSION
*Decrease of the total area of normal alveoles capable of gas exchange (pneumonia, lung edema) *Alveolar or capillary fibrosis (beryllium and asbestos exposure, silicosis)
DISTURBED RATE OF VENTILATION/PERFUSION
*Areas with reduced ventilation and maintained perfusion (emphysema) *Perfusion of unventilated alveoli (atelectasis) *Disturbances of pulmonary circulation due to shunts (Fallot's tetralogy)

MOUNTAIN SICKNESS

Although the symptoms threatening travelers crossing the mountain passes of Himalaya (4 - 5 km above sea level) were known from ancient times, the research on this topic begun only at the end of the XIXth century (Tab. 12.4). The study of this problem became very important during the 2nd World War because of the necessity of high-altitude flying of warplanes.

Mountain sickness is a special case of hypoxic hypoxia in some circumstances associated with hypobaria (Chapter 3). Its pathogenesis is slightly different in the case of mountain climbing (gradual decrease of oxygen tension and usually heavy physical burden) and in high-altitude flying (rapid

Tab. 12.4. The history of mountain sickness

ANCIENT TIMES - MIDDLE AGE Hui Jiao, a Chinese traveler, duke Mirza Muhammad Haidar and José Acosta, a Jesuitian monk independently from each other recognize the symptoms of the sickness affecting people dwelling in Himalaya and in the Andes mountains
NEW AGE 1644; E. Torricelli - air pressure measurements 1774; J. Priestly - discovery of oxygen 1789; A. Lavoisier - no life without oxygen 1783; The Montgolfiere brothers - hot air balloon flight
XIXth and XXth CENTURY 1878, 1879; P.Bert and A.Mosso first scientific studies on high altitude hypoxia 1930 - 1950 intensive military research on high altitude hypoxia 1953; Sir Edmund Hillary and Sherpa Tenzig conquer the Mount Everest (8848 m)

rise without acclimatisation). The following description is valid for untrained healthy persons. Well-trained persons after proper acclimatisation and people living in high altitude (e.g. Hans Messmer, the Sherpas from Himalaya mountain and the Ketchuas in the Andes) are able to live and work in very high altitudes even without extra oxygen supply. On the other side in persons with disturbed respiration and circulation the symptoms develop earlier than in healthy individuals.

The first symptoms sometimes occur already in altitudes of about 2.5 km above sea level but only in some (about 15%) individuals who ascend to this level quickly and without acclimatisation; the symptoms include headache, fatigue, nausea, dyspnoe and sleep disorders. Signs of pulmonary edema can develop after 2 - 3 days in some persons around 3 km meters and at about 3500 meters brain edema with psychomotoric syndrom threatens. Most of these symptoms are

not caused by hypoxia or hypoxemia but rather by compensatory mechanisms as cerebral vasodilatation (headache), pulmonary vasoconstriction (lung edema). The main problem in this type of hypoxia is that through compensatory hyperventilation **hypocapnia** and **respiratory alkalosis** develop and probably contribute to the disturbances of brain and pulmonary functions. In hypocapnia the actvity of respiratory centre is diminished and **periodic breathing** can occur.

A very dangerous phenomenon occuring even in well-trained and acclimatized persons is the **high-altitude euphoria**. The affected persons are behave as they were slightly drunk. They often underestimate the seriousness of situation (e.g. weather conditions, difficulties) and overestimate their own forces and abilities. This condition can be explained by the high sensitivity of the inhibitory functions of the cerebral cortex to hypoxia (a similar inhibition is seen in the first phase of alcohol intoxication). At higher altitudes gradual deterioration of the other cerebral functions - sensory, mental and motoric ensues. Above 5 - 6 km every single step requires enormous effort and psychical will.

12.3. ANEMIC HYPOXIA

Anemic hypoxia is caused by decrease of oxygen transport capacity of the blood (there is only a small proportion of oxygen physically dissolved in the blood fluids, the vast majority is bound to hemoglobin in red cells - Tab. 12.2). Insufficient transport function of hemoglobin can be caused by:
1/ **Insufficient amount of hemoglobin** in different types of anemia (see this chapter in Special Pathological Physiology).
2/ **Functional insufficiency of hemoglobin** (Tab. 12.5). In the

blood there is enough hemoglobin, but its oxygen transport capacity is blocked.

Tab. 12.5. Functional insufficiency of hemoglobin

Intoxication with carbon monoxide (CO)*
Oxidation of iron in the heme (methemoglobinemia) * strong oxidative stress (smoking, phenylhydrazine) * nitrate/nitrite intoxication in newborns * inborn hemoglobinopathies (M type) * deficit of methemoglobin reductase
Increased affinity of hemoglobin towards oxygen some inborn hemoglobinopathies, thalassemia alkalosis blockade of 2,3-BPG binding site (Hb glycation) decrease of 2,3-BPG concentration in the red cells

*Chapter 4.1

METHEMOGLOBINEMIA

Upon oxidation of divalent iron and binding of -OH moiety to hemoglobin **methemoglobin (metHb, HbOH)** arises which is worthless for oxygen transport. During normal circumstances the enzyme **methemoglobin reductase** (the enzyme exists in two forms working with coenzyme, NADH or NADPH, respcetively) continuosly reduces the oxidized hemoglobin and therefore in blood of healthy individuals only small concentration of metHb (0.1 - 0.4 % of total Hb) can be found. In heavy smokers not only the concentration of HbCO but also the concentration of MetHb (up to 8%) is elevated.

In patients with deficiency of **methemoglobin reductase** (type I or II) the concentration of metHb can rise to very high level. In other types of antioxidant system deficiency (e.g. in **glucose-6-phosphate deficency**, Chapter 6 and 8) he-

molysis occurs and therefore the hypoxia is caused not due to methemoglobinemia but as a consequence of anemia.

The oxygen binding site of Hb is at the bottom of a pocket lined with hydrophobic amino acid side chains. In some inborn defects of Hb structure (commonly named as "M" hemoglobins or **inborn methemoglobinemias**) the configuration of the pocket is faulty and as a consequence not only the O_2 molecule but also the bigger water molecule can get to the binding site. Interestigly enough, this immediately leads to iron oxidation and metHb formation.

A special case of **acquired** methemoglobinemia is the **infant nitrate methemoglobinemia**, an alimentary methemoglobinemia of artificially nourished infants (up to 2 months of life). It occurs in the case of high concentration of **nitrates** (>100 mg/l) in the drinking water. Nitrate from water used to prepare the infant milk formula is reduced by intestinal bacteria to **nitrite** and after resorbtion into blood oxidize the Hb in the red cells of infants whose antioxidant systems is not yet fully developed. Only very high concentration of nitrate in water cause measurable methemoglobinemia in adults.

In severe methemoglobinemia the colour of blood is from dark red to brown. The skin of the patients looks cyanotic with an ashy taint but in this case the proportion of reduced Hb is not elevated and the symptom is called **pseudocyanosis**.

HIGH OXYGEN AFFINITY OF BLOOD AS CAUSE OF HYPOXIA

The sigmoid shape of oxygen dissociation curve of hemoglobin is a very important prerequisite of oxygen release in the tissues. The difference is evident when the properties of myoglobin and hemoglobin are compared. At high oxygen concentration both proteins bind oxygen almost up to 100 %

saturation. In the tissues, however, the hemoglobin is able to release considerably more oxygen than the one-subunit myoglobin displaying a simple hyperbolic dissociation curve.

The sigmoidity of the hemoglobin dissociation curve is maintained by two different mechanisms:

* **Through cooperation between the subunits of hemoglobin when they switch from high- to low-affinity configuration and vice versa** (Fig. 12.1).

* **Through the effect of 2,3-bisphosphoglycerate on the dissociation curve.** This compound, ([2,3-BPG], a specific, and from energetic point of view unnecessary by-product of red cell glycolysis) binds to the terminal aminogroup of the β-chain of hemoglobin stabilizing it in the low affinity state. Similar effect on hemoglobin exert the increase of H^+ (acidosis) and CO_2 concentration (Bohr effect), (Fig. 12.2).

Every shift of the curve to the **right** (elevated oxygen affinity) makes the release of the oxygen in the tissues more difficult and leads to hypoxia without hypoxemia.

These conditions include some **hemoglobinopathies** (with disturbed cooperativity between subunits or fixed high-affinity configuration of the subunits). These hemoglobinopathies may but need not be associated with hemolytic anemia. In thalassemia and some other pathological conditions the concentration of fetal hemoglobin (F Hb) is elevated and this type of hemoglobin displays higher oxygen affinity as compared with Hb A (what is advantageous during intrauterine life). In glycated hemoglobin (Hb A_{1c}, Chapter 6.1) the 2, 3-BPG binding site is blocked by glucose and has (in vitro) increased oxygen affinity. In severe hypophosphatemia and is

some inborn disturbances of red cell glycolysis the concentration of 2,3-BPG can be abnormally low.

12.4. CIRCULATORY HYPOXIA

Circulatory hypoxia develops if the supply of blood to tissues is insufficient while the blood is normally oxygenated. Ithas two forms:

* **Total** and **local circulatory hypoxia**

Total circulatory hypoxia occurs at **heart failure or at shock**. Hypoxia of tissues is the consequence of insufficient left ventricular output, vasoconstriction (centralization of circulation) and stagnation of blood before an obstacle (right heart failure). There is no hypoxemia in (pure) circulatory hypoxia - the concentrations of Hb and pO_2 are normal. The extraction of oxygen by tissues is increased but because of insufficient supply of oxidezed blood the local pO_2 decreases to low values and pCO_2 is increased. In actual clinical situations the circulatory hypoxia is usually associated with other types of hypoxia - e.g. in left heart failure the exchange of gases in lungs is disturbed and in right heart failure the perfusion of lungs is decreased (**circulatory + hypoxic hypoxia**), in hemorrhagic shock the amount of Hb decreases and in burn shock the hemoglobin may be blocked by carbon monoxide (**circulatory + anemic hypoxia**).

Local circulatory hypoxia may be a consequence of arterial thrombosis (e.g. **myocardial infarct**), embolism, strong vasoconstriction or venous stagnation of blood.

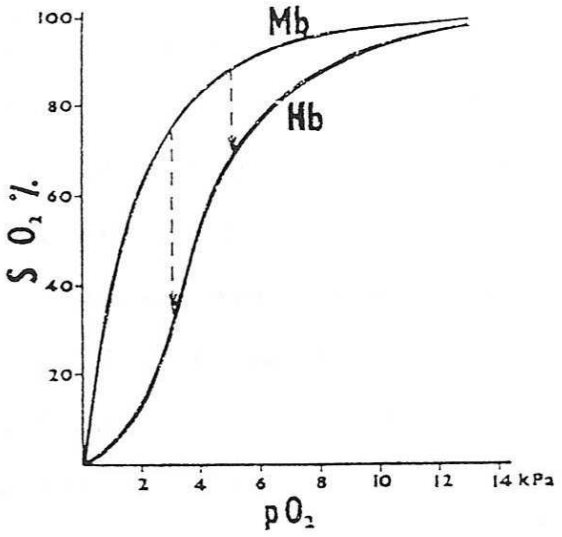

Fig. 12.1. The dissociation curves of myoglobin and hemoglobin.

At high oxygen concentration both myoglobin and hemoglobin are saturated with oxygen almost completely. In the tissues (pO_2 around 3 - 5 kPa hemoglobin is able to release considerable more oxygen than myoglobin. The difference is a consequence of sigmoid shape of the hemoglobin dissociation curve as compared with the hyperbolic dissociation curve of myoglobin. Every shift of the curve to the right (2,3-BPG, CO_2, acidosis) increases the release of the oxygen from hemoglobin. Left shift of the curve (alkalosis) deteriorates the oxygen release.

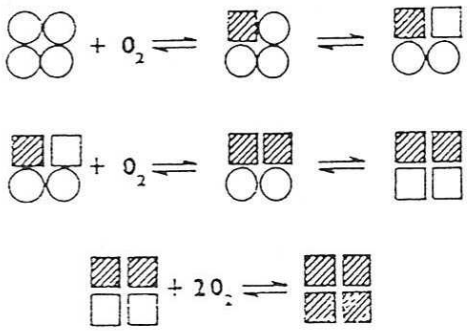

Fig. 12.2. The cooperation between subunits in the molecule of hemoglobin.

The subunits can exist in two configurations: circles represent the low and squares the high affinity state. The binding of first molecule of oxygen alters not only the conformation of this subunit (full squares) but induces a change in conformation of the second, which switches into high affinity state. In tissues the cooperation works in the opposite direction. The release of the first oxygen molecule helps to change the conformation of the others to the low affinity state and the 2nd - 4th oxygens are released very easily.

12.5. HISTOTOXIC HYPOXIA

It originates when the cells are not able to utilize oxygen, that is there is a disturbance of the mitochondrial **terminal oxidation** while there is sufficient oxygen in arterial blood. This hypoxia is not preceeded by hypoxemia. We can observe it especially in the case of intoxication with cyanides (Chapter 4.1) which block cytochromoxidase. It also occurs at damaging the enzymes of Krebs cycle with monobromacetone or tetrachlormetane and at overdose of anesthetics which on the other hand interfere with the system of dehydrogenases.

12.6. THE MAIN SYMPTOMS OF HYPOXIA

CYANOSIS

The basic symptom of chronically developing hypoxia is cyanosis. It is a term for blue or bluish colouring of skin, mucosae or even of the inner organs. It occurs when the concentration of reduced hemoglobin in capillary blood reaches 50 g/l. One can observe it most conveniently on the nail bed, on the lips and ears, nose, face, hands and feet.

There are two basic forms of cyanosis:

* central and peripheric cyanosis

Central cyanosis (arterial, anoxic) sevelops when the arterial reaching the tissues is not sufficiently saturated with oxygen and contains a certain amount of reduced hemoglobin. It occurs at inborn heart failures with right to left shunts (Fallot's tetralogy) and in different pulmonary diseases.

In **peripheric cyanosis** (acral, venous, stagnational) the arterial blood is usually normally saturated with oxygen but its flow through tissues is slowed down and therefore the ex-

traction of oxygen is increased. It is one of the symptoms of heart failure accompanied with prolongation of circulation time.

Local stagnational cyanosis ensues in the case of venous stagnation as a consequence of local processes - thrombosis, pressure of tumours, vasospastic diseases.

In mild cold environment cyanosis may manifest even in healthy persons on exposed locations because of constriction of skin arterioles and venules. The blood flow through capillaries is highly slowed down and more O_2 is removed from the blood. In strong cold environment, however cyanosis does not develop because the drop of skin temperature prevents the dissociation of hemoglobin and the consumption of O_2 in subcooled tissues is reduced.

In decompensated cor pulmonale chronicum and at chronical left heart failure the cyanosis is **combined (insufficient saturation of Hb + circulation failure)**.

Cyanosis does not develop in anemia because at low total concentration of Hb (e.g. 90 - 60 g/l) it is difficult to reach the deoxyhemoglobin concentration required for the manifestation of cyanosis (50 g/l). On the other side cyanosis easily develops in polycythemic conditions. There is no cyanosis in carbon monoxide intoxication (the colour of HbCO is light red) and at histotoxical hypoxia since pO_2 and Hb saturation are normal here.

As above already mentioned, cyanosis-like colour of skin and mucosae can be also caused by high levels of methemoglobin in the blood.

DYSPNOE

The second most frequent clinical symptom of hypoxia is

dyspnoe. It is hardened breathing with subjective feeling of air shortage. It can be characterized by anxious expression of face, accelerated breathing, deepening of breathing excursions, increased work of auxiliary respirataory muscles and movement of nostrils.

Dyspnoe arises when ventilation is increased 4-5 times as compared with ventilation at rest. At mild hypoxia there is first tachypnoe (accelerated breathing) and hyperpnoe (deepened breathing), dyspnoe occurs only later. Another type of dyspnoe occurs with left side heart failure
(due to pulmonary congestion -> Special Pathological Physiology).

These symptoms are often joined by general lassitude, fatigue at minimal exercise and symptoms from the side of nervous system including sleep disorders. Another group of symptoms arise as a consequence of compensatory measures.

12.7. MECHANISMS OF COMPENSATION IN HYPOXIC CONDITIONS

The oxygen supply of the body is assured by respiratory system, blood, cardiovascular system and tissues themselves. At damage of any of these mechanisms **all undisturbed systems or their intact parts join in the compensation.**

Hyperventilation (tachypnoe and hyperpnoe) is efficient in most cases of hypoxic hypoxia but appears in anemic hypoxia, too. Dyspnoe is also a compensatory mechanisms but the maximal effort of repsiratory muscles can consumes a lot of oxygen and therefore the overall balance is often negative.

Tachycardia is an important compensatory mechanism both

in hypoxic and anemic hypoxia, but it consumes oxygen, too. In hypoxic condition (e.g. shock, severe anemia) **centralization** of circulation develops. The objective of this mechanism is to maintain the blood and oxygen supply to the most important organs (heart, brain) at the expense of circulation of the skin, muscles and inner organs. In acute conditions it can save the life but after some hours irreversible organ damage (kideny, liver) develops and can lead to death even in the case of restoration of the circulation.

The activation of the renin-angiotensin-aldosetron system is advantageous in hypovolemic conditions (blood loss) but is harmful in circulatory hypoxia.

Erythropoetin is another kidney hormone released in every hypoxic condition. As the main regulatory factor of erythropoesis, erythropetin increases the production of red cells with the exception of some forms of anemia. **Polycythemia** is useful after blood loss but harmful (increased blood viscosity) in pulmonary and heart disease.

Better release of oxygen from blood in tissues is also possible due to elevated concentration of 2,3-bisphosphoglycerate and elevated concentration of CO_2 in blood and tissues (shift in the dissociation curve of the hemoglobin to the left.

In hypoxemia the tissues themselves are also capable to increase the oxygen extraction from blood.

12.8. MANIFESTATION OF HYPOXIA IN SOME ORGANS AND TISSUES

Brain tissue uses about 20 % of total oxygen consumption

and is exceptionally sensitive to hypoxia. The most sensitive structure of CNS is the cerebral cortex. Therefore at heavy acute hypoxia (e.g. at heart stop) in the course of some seconds general seizures and unconsciousness appears. In slight hypoxia the permeability of cerebral capillaries increases and that may lead to cerebral edema.

At slow development of brain hypoxia gradual intellectual deterioration, headache, weakness of the memory, sleep disturbances and various neurological disturbances are the main symptoms.

The most sensitive structure of **heart** which uses 15 % of total oxygen consumption is the conductive system. Therefore at hypoxia there is a frequent increased irritability of conductive system what can result in various dysrhytmias. At chronic hypoxia there may arise also heavy structural changes - fibrosis of the heart muscle, hypertrophia and dilatation of the heart. As heart is one of the most imprtant part of the oxygen supplying system, a vicious circle develops which even more deteriorates the oxygen supply to myocard.

In **lungs** hypoxia causes vasoconstriction of small pulmonary veins. The pulmonary vascular resistance is increased what increases the work of right heart.

Hypoxia of **kidneys** at acute renal ischemia causes structural and functional changes which can lead even to renal insufficiency (e.g. at a shock. In hypoxic kidneys is the synthesis and release of erythropoetin and activitedrenin renin is increased.

In **liver** even at normal circumstances the cells located in the centre of liver lobe receive a limited dose of oxygen. It results from the structure of functional and nutritional

circulation of the liver. Therefore in pathological conditions these centrilobular cells suffer as first. Centrilobular necrosis and consequent fibrosis can easily develop.

Hypoxia in skeletal musculature may occur even at excessive muscle activity when muscles gain energy through anaerobic glycolysis. If during the unadequate supply of oxygen the muscles are not able to acquire sufficient amount of ATP through glycolysis, painful contractures may appear.

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