Zusammenfassung. In der vorliegenden Arbeit wird ein Überblick über die Probleme gegeben, die bei der Interpretation der Wirkung von Substanzen auftreten, welche die passive Ionenpermeabilität der Erythrozytenmembran beeinflussen. Dabei wird besonderes Gewicht auf Hemmstoffe der Anionenpermeabilität gelegt.

1-Fluoro-2,4-Dinitrobenzol (DNBF) und 5-Methoxy-2-Nitrotropon (MNT) können mit Aminogruppen kovalente Bindungen eingehen. Beide Substanzen werden von der Erythrozytenmembran irreversibel gebunden. Sie hemmen die Permeabilität für Anionen und steigern sie für Kationen. Die Wirkung des MNT auf die Kationenpermeabilität wird allerdings nur sichtbar, wenn es zusammen mit einer nahezu hämolytischen Menge an Äthanol dem Inkubationsmedium zugesetzt wird. Die Beobachtungen stehen im Einklang mit der Hypothese, dass die Erythrozytenmembran ein Anionenaustauscher ist, in dem Aminogruppen als Träger der positiven Festladungen vorhanden sind.

Schwieriger lässt sich die Hemmwirkung einer Reihe von Stoffen erklären, die nicht mit Aminogruppen reagieren können. Dinitrophenol und Benzoat hemmen den Sulfationenfluss nichtkompetitiv, ohne dabei eine Steigerung des Kaliumeffluxes herbeizuführen. Auch aliphatische Verbindungen, darunter primäre Alkohole und Amine, können die Anionenpermeabilität vermindern, wobei bereits ein deutlicher Hemmeffekt auftritt, bevor der Kaliumefflux vergrössert wird. Die einzige Gemeinsamkeit dieser chemisch sehr heterogenen Gruppe an Hemmstoffen ist der polar-apolare Charakter ihrer Moleküle, was zu einer Anreicherung dieser Stoffe an Grenzflächen zwischen Lipid und Wasser führen sollte.

Die Beziehung zwischen Sulfatpermeabilität und Hemmstoffkonzentration lässt sich mit Hilfe einer einfachen, aus der Enzymkinetik bekannten Formel beschreiben. Zwischen dem gemessenen Sulfatfluss und der aufgrund der Festladungshypothese berechneten Sulfatkonzentration in der Membran besteht ein exponentieller Zusammenhang, der durch eine empirische Gleichung wiedergegeben werden kann. Mit Hilfe dieser Gleichung lässt sich der Anionenfluss auch in Gegenwart eines Hemmstoffes (Phenol) beschreiben, wobei sich nur eine der beiden empirischen Konstanten erheblich ändert.

Die geschilderten Ergebnisse legen die Vermutung nahe, dass die Anionen bei der Penetration durch die Erythrozytenmembran mindestens zwei verschiedene, hintereinander geschaltete Permeabilitätsbarrieren überwinden müssen.

Hemoglobin

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Hemoglobin is a spheroidal molecule of 65 by 55 by 50 Å in diameter[1] and contains 2 unequal polypeptide chain pairs with a heme linked to each chain. During human life 4 different hemoglobins are formed: the embryonic Hb Gower 2, the fetal Hb F and the 2 adult hemoglobins Hb A and Hb A_2. They differ in one polypeptide chain pair: Hb A = $\alpha_2\beta_2$, Hb F = $\alpha_2\gamma_2$, Hb A_2 = $\alpha_2\delta_2$ and Hb Gower 2 = $\alpha_2\varepsilon_2$. The α-chain contains 141, each of the other chains 146 amino acids[1a]. Recently SCHROEDER et al.[2] described 2 types of Hb F in normal infants with glycine and alanine respectively in position 136 of the γ-chains. In all polypeptide chains an identical heme is situated near the surface between 2 helices of the chain[1,3]. Out of the 6 coordination bonds of the heme-Fe^{++}, 4 are bound to the porphyrin ring, the 5th is linked to the so-called proximal histidine α^{87} and β^{92} respectively and the 6th is located opposite the distal histidine α^{58} and β^{63} and represents the site of reversible combination with molecular oxygen. In deoxyhemoglobin the oxygen is replaced by a water molecule and the distance between the Fe^{++} atoms of both β-chains is increased from 33.4 to 40.3 Å[4]. The heme-heme interaction resulting in the sigmoid shape of the oxygen dissociation curve requires 2 unequal polypeptide chain pairs. During red cell aging a slight increase of the O_2-affinity of the hemoglobin and a decrease of the heme-heme interaction occur, which are attributed to an alteration of electrostatic interactions in the molecule[5].

Besides the normal hemoglobins an increasing number of abnormal human variants have been described, designated first by Latin capital letters and later by proper names. At the present time more than 70 ab-

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normal hemoglobins have been chemically analyzed. They can be classified in groups of different structural abnormalities. The large majority of abnormal variants differ from the corresponding normal hemoglobin in a single amino acid of one of the two types of polypeptide chains. In all cases this substitution can be attributed to a single base mutation in the genetic code[6]. Single amino acid substitutions occur in the α-, β-, γ- and δ-chains (e.g. Hb I = $\alpha_2^{16\,Lys\rightarrow Glu}\,\beta_2$, Hb S = $\alpha_2\,\beta_2^{6\,Glu\rightarrow Val}$, Hb F Texas I[7] = $\alpha_2\,\gamma_2^{5\,Glu\rightarrow Lys}$ and Hb Flatbush[8] = $\alpha_2\,\delta_2^{22\,Ala\rightarrow Glu}$). Abnormal α-chains produce abnormal variants of Hb F, Hb A and Hb A_2, while anomalies of the other chains result in the production of a single abnormal hemoglobin. One abnormal hemoglobin makes an exception, being due to a double mutation with substitution of 2 amino acids in the same polypeptide chain: Hb C Harlem[9,10] = $\alpha_2\,\beta_2^{6\,Glu\rightarrow Val,\ 73\,Asp\rightarrow Asn}$. The first substitution is identical with that in Hb S and the second has been described in Hb Korle-Bu[11].

In other types of abnormal hemoglobins one or more amino acids are missing in one polypeptide chain. By deletion the amino acid β23 Val is missing in Hb Freiburg[12]. The deletion of 5 sequential amino acid residues in the heme binding region of the β-chains with lack of the corresponding heme has been described in Hb Gun Hill[13,14]. Such an anomaly can be produced by unequal crossing over. Moreover, an acquired abnormal hemoglobin with one amino acid missing has been found: Free Hb A in normal plasma is converted to Hb Koelliker, which has lost the C-terminal α 141 Arg[15].

A different type of abnormality is found in the Hb Lepore-variants. Hb Lepore contains 2 normal α-chains which correspond in the N-terminal part to normal δ- and in the C-terminal part to normal β-chains. The point of fusion is between β 22–50 in Hb Lepore Hollandia and between β 50–116 in Hb Lepore Washington. An exact location is impossible, 138 out of the 146 amino acid positions being identical in β- and δ-chains. Hb Lepore is attributed to unequal crossing over of the δ- and β-genes with deletion of the missing parts[16]. From this it can be concluded, that the 2 genes are located close together in the same chromosome.

Yet an other type of hemoglobin anomaly to be mentioned comprises the tetramers formed by 4 identical polypeptide chains. Tetramers with normal chains are Hb H = β_4, Hb Bart's = γ_4 and probably Hb Gower 1 = ε_4. Recently Hb α_4 was found under special conditions[17]. Tetramers with abnormal chains are Hb Augusta I[18] = $\beta_4^{6\,Glu\rightarrow Val}$ and Hb Augusta II[18] = $\beta_4^{6\,Glu\rightarrow Lys}$, the former containing the anomaly of Hb S and the latter the substitution of Hb C. Finally a unique hemoglobin without α-chains has been reported as Hb Portland 1[19] which is thought to consist of 2 γ- and 2 ε-chains.

The relations between structural abnormality and functional disturbance represent one of the most attractive problems of present hemoglobin research. The majority of abnormal hemoglobin variants are functionally normal, i.e. the anomaly causes no physiologic disturbance. Other hemoglobins may under given conditions be alterated or denaturated and precipitated within the red cell. The water solubility of deoxygenated Hb S ($\alpha_2\,\beta_2^{6\,Glu\rightarrow Val}$) is 25 times less than that of deoxygenated Hb A. In erythrocytes with high Hb S content deoxygenated Hb S precipitates and forms tactoid crystals growing to a length of up to 15 μ, which deform the cell membrane and are responsible for the sickling phenomenon. Spectropolarimetric measurements by MURAYAMA[20] suggest, that the reduced solubility is due to hydrophobic interaction between the N-terminal Valyl and the new Valyl β_6 forming a ring structure of the N-terminal part of the polypeptide chain. The intracellular structure of the precipitated Hb S can be demonstrated by electron microscopy[21,22]. A similar sickling phenomenon is caused by Hb C Harlem[9] and has been described in a case with Hb I ($\alpha_2^{16\,Lys\rightarrow Glu}\,\beta_2$)[23]. Likewise Hb C ($\alpha_2\,\beta_2^{6\,Glu\rightarrow Lys}$) is known to form intraerythrocytic hemoglobin crystals[24].

Several abnormal hemoglobins impair the red cell function by their molecular instability. In the Hb M-mutants the abnormal heme function is due to a substitution of the distal histidin (e.g. Hb M Boston[25] = $\alpha_2^{58\,His\rightarrow Tyr}\beta_2$, Hb M Saskatoon[25] = $\alpha_2\,\beta_2^{63\,His\rightarrow Tyr}$) the proximal histidin (e.g. Hb M Iwate[26] = $\alpha_2^{87\,His\rightarrow Tyr}\,\beta_2$) or to an other substitution in the region of the heme

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(Hb M Milwaukee I[25] = $\alpha_2\ \beta_2^{67\,Val\rightarrow Glu}$). The Hb M variants are present in the form of methemoglobin and are therefore unable to bind oxygen reversibly. Other abnormal hemoglobins with an unstable globin undergo oxydative denaturation and form Heinz bodies, a process leading to premature lysis of the red cells and therefore to hemolytic anemias. Such unstable hemoglobin variants are Hb Zürich[27] ($\alpha_2\ \beta_2^{63\,His\rightarrow Arg}$), Hb Köln[28,29] ($\alpha_2\ \beta_2^{98\,Val\rightarrow Met}$), Hb Hammersmith[30] ($\alpha_2\ \beta_2^{42\,Phe\rightarrow Ser}$), Hb Sydney[31] ($\alpha_2\ \beta_2^{67\,Val\rightarrow Ala}$) Hb Genova[32] ($\alpha_2\ \beta_2^{28\,Leu\rightarrow Pro}$) and Hb H ($\beta_4$).

During the last years a number of abnormal hemoglobin variants with increased oxygen affinity have been described. They impair the red cell function by impeding deoxygenation in the peripheral circulation, which results in a compensatory erythrocytosis. To this group belong Hb Chesapeake[33] ($\alpha_2^{92\,Arg\rightarrow Leu}\ \beta_2$), Hb Yakima[34,35] ($\alpha_2\ \beta_2^{99\,Asp\rightarrow His}$), Hb Rainier[36] ($\alpha_2\ \beta_2^{145\,Tyr\rightarrow His}$), Hb Kempsey[37] ($\alpha_2\ \beta_2^{99\,Asp\rightarrow Asn}$), Hb J Cape Town[38] ($\alpha_2^{92\,Arg\rightarrow Gln}\ \beta_2$), Hb Hiroshima[39] ($\alpha_2\ \beta_2^{143\,His\rightarrow ?}$), Hb Ypsi[40] (structural β-alteration not yet reported) and Hb H (β_4).

In summary it may be concluded that the majority of hemoglobin anomalies have no functional implications, some produce methemoglobinemia by impaired heme stability, some lead to hemolytic anemias by decreased solubility or by globin instability and others cause erythrocytosis by increased oxygen affinity.

Zusammenfassung. Kenntnisse über die Struktur des Hämoglobinmoleküls bilden den Ausgangspunkt zum Verständnis abnormer Blutfarbstoffvarianten. Anomalen Hämoglobinen liegen verschiedene Strukturanomalien zugrunde: Substitution oder Deletion einer oder mehrerer Aminosäuren in einer Polypeptidkette, durch verschiedenartiges «Crossing over» entstandene anomale neue Polypeptidketten oder eine ungewöhnliche Kombination an sich normaler Polypeptidketten. Bei zahlreichen anomalen Varianten lassen sich funktionelle Besonderheiten aus der Strukturanomalie ableiten. Funktionsanomalien kommen durch verminderte Löslichkeit, gesteigerte Oxydierbarkeit des Hämeisens, herabgesetzte Globinstabilität gegenüber oxydativen Noxen oder durch erhöhte Sauerstoffaffinität zustande. Ihre klinischen Folgen sind hämolytische Anämien, Methämoglobinämien und kompensatorische Polyglobulien.

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Biochemistry of the Erythrocyte

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The primary function of the human erythrocyte is to deliver oxygen to the tissues and to carry carbon dioxide from the tissues to the lungs. These functions do not in themselves, require the expenditure of metabolic energy. However, to perform them efficiently, it is necessary for the red cell to carry a highly concentrated solution of hemoglobin while preserving the biconcave form of the cell. It must protect the membrane and the hemoglobin from oxidative damage and prevent osmotic hemolysis. Preservation of the constituents of the red cell in an active form and the maintenance of ionic gradients across the cell membrane require a source of metabolic energy.

Under artificial conditions, the red cell is relatively versatile in the range of substrates from which it can

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