(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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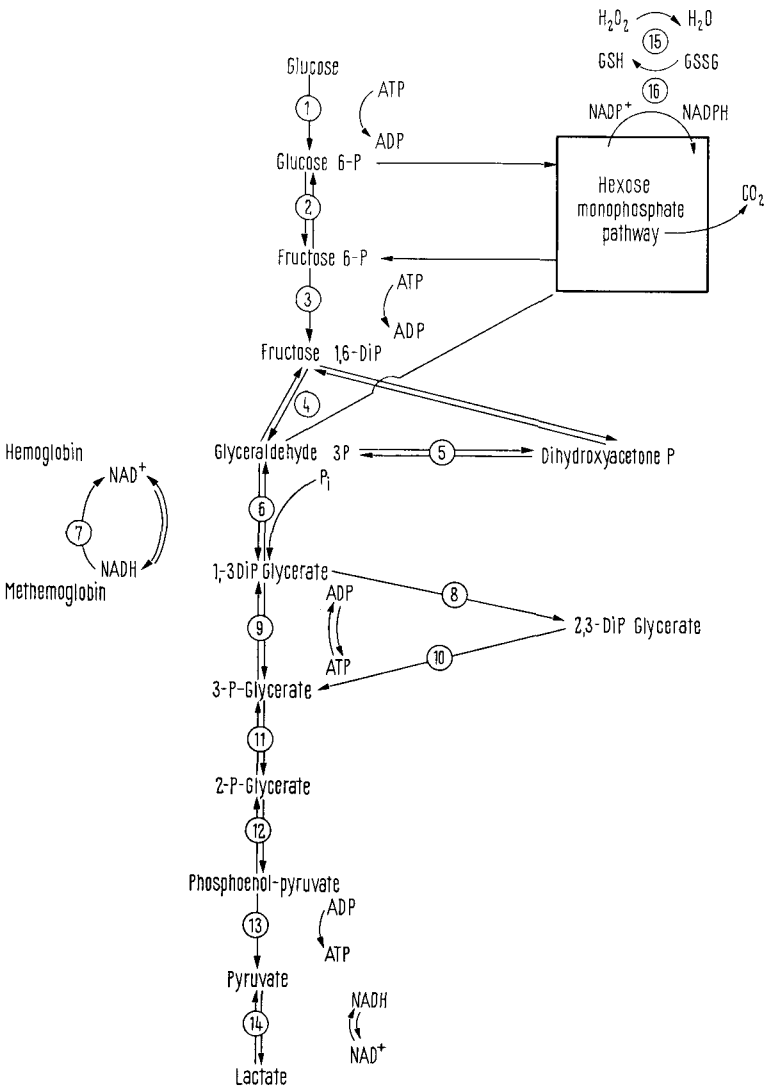
extract energy. It can metabolize fructose, mannose, inosine or adenosine with considerable ease, is able to use galactose to a limited extent, and can even use relatively esoteric compounds such as formaldehyde as a source of energy under appropriate conditions. In the circulation, however, it is undoubtedly glucose which forms the main sustenance of the erythrocyte.

The red cell possesses a metabolic mechanism which seems well suited to its requirements for the conversion of energy of the glucose molecule to energy in the form of ATP and reduced pyridine nucleotides. This metabolic machinery differs from that of most other tissues because the mature erythrocyte has no intracellular organelles. It has no nucleus to direct RNA synthesis and no ribosomes to permit the synthesis of new protein molecules to replace those that have been worn out. It has no mitochondria to produce relatively large amounts of ATP from small amounts of carbohydrates. Rather, the erythrocyte is dependent upon those enzymes which are contained within its membrane at the time of its maturation from the reticulocyte, and must depend upon anaerobic glycolysis and the hexose monophosphate pathway as a source of metabolic energy. It is perhaps somewhat anomalous that the red cell, with its relatively huge load of oxygen, diverts only minute quantities of the gas for its own use. Most of the metabolism of the red cell is anaerobic.

Before glucose can be utilized, it must pass through the erythrocyte membrane. This process, which has been the object of intensive, fruitful study, is described elsewhere in this volume. But the entry of glucose into the red cell is not enough. It must be phosphorylated to glucose-6-phosphate before it can serve as a source of energy for the cell. This process is mediated by hexokinase and requires expenditure of energy in the form of ATP. Once glucose has been phosphorylated it can traverse either one of 2 pathways. Under normal circumstances, over 90% of the glucose is metabolized



Glucose metabolism of the red cell. The reactions indicated are catalysed by the following enzymes: (1) hexokinase; (2) glucosephosphate isomerase; (3) phosphofructokinase; (4) aldolase; (5) triosephosphate isomerase; (6) glyceraldehydephosphate dehydrogenase; (7) methemoglobin reductase; (8) diphosphoglyceromutase; (9) phosphoglycerate kinase; (10) diphosphoglycerate phosphatase; (11) phosphoglyceromutase; (12) enolase; (13) pyruvate kinase; (14) lactate dehydrogenase; (15) glutathione peroxidase; (16) glutathione reductase.

by way of the Embden-Meyerhof pathway (Figure) in which it is catabolized to lactate or pyruvate. Less than 10% passes through the direct oxidative shunt where catabolism to CO_2 as well as pyruvate occurs. The Embden-Meyerhof pathway of the red cell provides it with a means of phosphorylating adenosine diphosphate (ADP) to adenosine triphosphate (ATP). A source of ATP is needed, not only to make glucose available for further metabolism but also to serve as the main source of energy for some of the ion pumps which operate in the red cell membrane. The Embden-Meyerhof pathway also provides a means by which nicotinamide adenine dinucleotide (NAD^+) is reduced to reduced nicotinamide adenine dinucleotide (NADH). It is this coenzyme which provides the reducing power which makes it possible for the erythrocyte to maintain the iron of its hemoglobin in the ferrous state. This is a vital function of the enzyme, methemoglobin reductase. If there were no means of reducing ferrihemoglobin (methemoglobin) to ferrohemoglobin (reduced hemoglobin) red cells would gradually turn brown and fail to carry oxygen. But in the presence of an efficient methemoglobin reducing system, methemoglobin levels in the erythrocyte are normally maintained at well under 1%.

It is also necessary for the erythrocyte to maintain sulfhydril (–SH) groups found in the hemoglobin, enzymes and in the red cell membrane in their active, reduced, form. This seems to be a function of the tripeptide, glutathione (GSH). In the process of reducing –SH groups in red cells GSH is oxidized to oxidized glutathione (GSSG). This disulfide can be actively extruded from the red cell or can be reduced back to GSH by the enzyme systems of the red cell. The reduction of GSSG is achieved chiefly through mediation of the enzyme, glutathione reductase, which preferentially utilizes reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a hydrogen donor. This coenzyme is maintained in the reduced form in metabolism of glucose-6-phosphate through the direct oxidative pathway. Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is reduced both in the glucose-6-phosphate dehydrogenase and phosphogluconic dehydrogenase steps.

A remarkable and admirable feature of erythrocyte metabolism is the flexibility of the relatively few metabolic pathways which are available. For example, the Rapoport-Luebering cycle provides an 'energy clutch' for the erythrocyte, enabling the cell to vary the amount of ADP phosphorylated to ATP without any alteration in the rate of glucose metabolism and NAD reduction. If 1,3-diphosphoglycerate is converted to 2,3-diphosphoglycerate in the diphosphoglyceromutase reaction, no ADP is phosphorylated to ATP. In contrast, metabolism of 1,3-diphosphoglycerate directly to 3-phosphoglycerate through the phosphoglycerate kinase reaction results in the phosphorylation of ADP to ATP. Similarly, there is flexibility in the amount of NAD^+ reduced to NADH. In the presence of large amounts of methemoglobin, NADH formed in the glyceraldehyde phosphate dehydrogenase step may be oxidized entirely in the course of methemoglobin reduction. Pyruvate then represents the end stage of glucose metabolism by the erythrocyte. If no NADH is required for methemoglobin reduction, on the other hand, pyruvate will be reduced to lactate in the lactate dehydrogenase reaction. The red cell also has the capacity to regulate the amount of $NADP^+$ reduced to NADPH, since the level of $NADP^+$ within the erythrocyte appears to be an important limiting factor in regulating the rate of the glucose-6-phosphate dehydrogenase reaction. Thus, when an increased requirement for NADPH arises, as may occur during the administration of drugs which oxidize glutathione, the rate of metabolism by way of the direct oxidative shunt automatically increases.

Until recently, the level of metabolic intermediates within the erythrocyte has seemed to be of interest primarily in the study of the control of the rate of metabolism of the red cell. Recently, however, it has been found that an important relationship also exists between the level of certain metabolic intermediates within the cell and the primary function of the erythrocyte, namely, its capacity to deliver oxygen to the tissues. Specifically, 2,3-diphosphoglycerate and ATP are both bound by hemoglobin, and markedly alter its affinity for oxygen. In the presence of these compounds, hemoglobin binds oxygen less avidly, and the oxygen dissociation curve therefore shifts to the right. When these phosphorylated compounds are depleted from the red cell on the other hand, a marked shift of the dissociation curve to the left is observed.

The energy producing system of the red cell is vital in providing a source of energy for methemoglobin reduction and for active transmembrane transport. However, the erythrocyte also contains a number of other enzymes. For example, the complete series of enzymes required for the metabolism of galactose, galactokinase, galactose-1-phosphate uridyl transferase, epimerase, and phosphoglucomutase can be found in the erythrocyte. Enzymes of glycogen metabolism are also present. In the membrane various ATPase, which have important functions in ion transport and acetylcholinesterase, the function of which is obscure, may be found. The red cell is a very rich source of catalase. Mutarotase, acid phosphatase, carbonic anhydrase, inosine phosphorylase, glutathione synthetase, adenylate kinase, hypoxanthine-guanine phosphoribosyltransferase, and even amino acid activating enzymes are found in the hemolysates prepared from erythrocytes.

The many enzymes and metabolic pathways which exist in the red cell, and its easy availability in relatively homogenous suspensions make the red cell an admirable model for the study of genetic variation, metabolic control, and for trans-membrane phenomena.

Zusammenfassung. Der Erythrozyt hat zwei Hauptwege für den Glukoseabbau. Der Emden-Meyerhoff-Weg resultiert in der Phosphorylierung von ADP zu ATP und der Reduktion von NAD^+ zu NADH. Der Hexose-Monophosphat-Weg ermöglicht andererseits die Reduktion von $NADP^+$ zu NADPH. Es besteht eine beachtliche Beweglichkeit in der Kontrolle dieser Bahnen, die es dem Erythrozyten ermöglichen, seine Enzyme und sein Hämoglobin in aktiver Form und das Konzentrationsgefälle von Natrium und Kalium zwischen roten Blutkörperchen und Plasma zu erhalten.

SPECIALIA

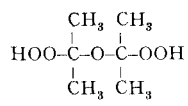
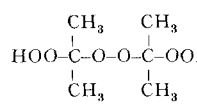
Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Ответственность за короткие сообщения несёт исключительно автор. - El responsable de los informes reducidos, está el autor.

Peroxides of Diisopropylether

Investigating the ozonisation of tetramethylethylene[1], it was found desirable to have for comparison IR-spectra and Rf values of some suitable simple peroxide containing, besides the hydroperoxy, also an ether group. Such a compound, 2,2'-dihydroperoxy-2,2'-diisopropyl ether (I), was originally prepared by IVANOV, SAVINOVA and MICHAILOVA[2]. When peroxide I, prepared according to IVANOV et al., was analysed by the paper chromatographic method of MILAS and BELIČ[3], 2 peroxydic spots in addition to H_2O_2 were detected. Further confirmation of the presence of 2 peroxides was obtained by thin layer chromatography which was successfully used for the separation of peroxides by several authors[4]. Best separation was obtained with silikagel G and a mixture of toluene and methanol as used by BUZLANOVA et al.[5]. With this method also, 2 peroxides could be detected. The Table gives the Rf values obtained by the above chromatographic methods.

With the aid of preparative thin layer chromatography, both peroxides have been isolated in pure state and the purity of both was checked by tlc. The peroxide with the higher Rf value, suspected on the basis of its molecular weight and active oxygen content to be 2,2'-dihydroperoxy-2,2'-diisopropyl peroxide (II), was found to have an IR-spectrum and Rf values which were identical with those of II prepared according to MILAS and GOLUBOVIČ from acetone[6]. Therefore, the peroxide with the lower Rf was held to be I. It had the expected molecular weight and active oxygen content and solidifyed when kept for a few days at room temperature. After crystallizing from pentane, this solid peroxide was identified as 1,1,4,4,7,7-hexamethyl-1,4,7-cyclononatriperoxane by its melting point, Rf value and IR-spectrum. Peroxide II remained unchanged under the same conditions. It is known that only peroxides containing an ether group rearrange spontaneously, whereas peroxides containing peroxy groups do so only in the presence of strong acids[1]. The IR-spectra of the separated peroxides I and II also support the above conclusion, peroxide I showing strong bands at 1.042 and 988 cm^{-1} which are absent in the IR-spectrum of II. Therefore, when a synthesis of peroxide I is attempted according to IVANOV et al. a mixture of I and II is obtained, as a consequence of which a chromatographic separation of pure I is necessary[7].

Rf values of the peroxides of diisopropyl ether

	Dimethyl-formamide-decaline (pc)	Butanol-ethanol-H_2O (pc)	Toluene-methanol (thin layer chromat.)
 I	0.01	0.75	0.24
 II	0.13	0.84	0.64

Zusammenfassung. Stellt man 2,2'-Dihydroperoxy-2,2'-Diisopropyläther nach IVANOV et al. aus Isopropyläther her, so entsteht nebenbei auch noch 2,2'-Dihydroperoxy-2,2'-Diisopropyl-Peroxid. Beide Peroxide kann man papier- und dünnschichtchromatographisch nachweisen und mit präparativer Dünnschichtchromatographie rein isolieren.

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