Zusammenfassung. In Gegenwart des Chelatbildners Dipyridyl ist die unter der Einwirkung von UV-Bestrahlung eintretende Oxydation von Prolin zu Hydroxyprolin gehemmt, wie hier am Beispiel von neutralsalzlöslichem dermalem Kollagen des Menschen gezeigt werden konnte. Die unter den gleichen Bedingungen einsetzende Degradation der primären Kollagenketten wird jedoch durch die Gegenwart von Dipyridyl nicht gestört, so dass ein direkter Zusammenhang zwischen den beiden Effekten ausgeschlossen werden kann.

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The Effect of Anaerobic Incubation Upon 2,3-Diphosphoglycerate Synthesis in vitro

It has been observed that 2,3-diphosphoglycerate (2,3-DPG) binds with greater affinity to reduced than to oxygenated adult human hemoglobin[1–4]. The binding of 2,3-DPG by deoxyhemoglobin within the adult human erythrocyte relieves 2,3-diphosphoglycerate mutase (D-1,3-diphosphoglyceric acid: D-3-phosphoglycerate phosphotransferase, EC 2.7.5.4) from inhibition by its product and facilitates further synthesis of 2,3-DPG[5]. While studying the effects of added deoxy- and carboxyhemoglobin upon 2,3-DPG synthesis, it was noted that the generation of 2,3-DPG from fructose-1,6-diphosphate was accelerated under nitrogen even in the absence of added reduced hemoglobin[6]. It is the purpose of this communication to describe the mechanism of this effect.

Hemoglobin-free enzyme mixture which contained red cell 2,3-DPG mutase and was free of 2,3-DPG phosphatase (D-2,3-diphosphoglycerate 2-phosphohydrolase, EC 3.1.3.13), was prepared according to the method described elsewhere[5]. Synthesis of 2,3-DPG was studied in an incubation medium containing red cell 2,3-DPG mutase; fructose-1,6-diphosphate, 100 μmoles; NAD, 20 μmoles; 3-phosphoglycerate, 10 μmoles; EDTA, 35 μmoles; triethanolamine buffer, pH 7.6, 360 μmoles; potassium disodium phosphate, 70 μmoles; aldolase (ketose-1-phosphate aldehyde-lyase, EC 4.1.2.7), 9 units; and glyceraldehyde-3-phosphate dehydrogenase (D-glyceraldehyde-3-phosphate: NAD oxidoreductase, EC 1.2.1.12, G-3-PD), 36 units; in a total volume of 10.5 ml. The generation of 2,3-DPG was studied in air, 100% nitrogen and 100% carbon monoxide. Incubation mixtures were allowed to equilibrate with the appropriate atmosphere for 30 min prior to the initiation of the reaction by the addition of fructose-1,6-diphosphate. Compressed air, nitrogen and carbon monoxide were washed via a fritted glass dispersion tube immersed in distilled water prior to delivery into the reaction mixture. One ml samples were removed periodically and deproteinized by immersion in boiling water. The supernatants were assayed for 2,3-DPG using the Schroter and Heyden[7] modification of the method devised by Krimsky[8].

The rates of synthesis of 2,3-DPG in incubation mixtures supplemented with enzyme protein containing adult red cell 2,3-DPG mutase are shown in Figure 1. The total synthesis of 2,3-DPG was approximately 18% greater in anaerobic incubation mixtures, maintained under either nitrogen or carbon monoxide, than in mixtures incubated in air. The increase in 2,3-DPG synthesis induced by anaerobic incubation was best observed at phosphate concentrations of 7 m*M* or greater. When phosphate was omitted from the reaction mixture only approximately 15% as much 2,3-DPG was synthesized and the differential effect of anaerobic incubation was not observed.

While 2,3-DPG synthesis appears to be a function of phosphate concentration, 2,3-DPG mutase is not known to be phosphate dependent[9]. However, G-3-PD is sensitive to phosphate concentrations[9,10]. G-3-PD activity

Effect of phosphate concentration upon glyceraldehyde-3-phosphate dehydrogenase activity

Phosphate concentration (m*M*)	Glyceraldehyde-3-phosphate reduced after 1 h (nmoles/ml)
0	32
0.7	39
3.5	58
7.0	72

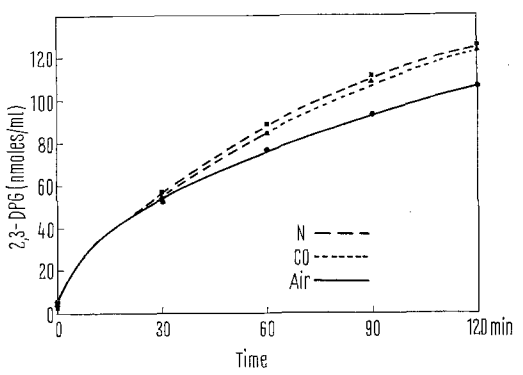

Fig. 1. The rate of 2,3-DPG synthesis in incubation mixtures maintained under air, carbon monoxide and nitrogen. Each point represents the average value of 5 experiments. See text for experimental details. In these experiments phosphate final concentration was 7 m*M*.

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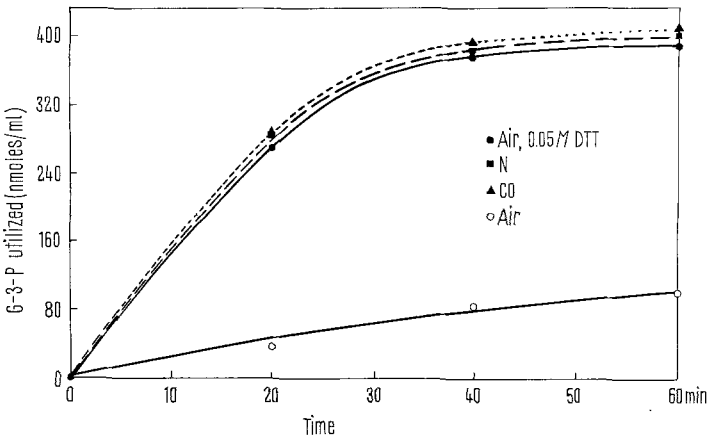

Fig. 2. Glyceraldehyde-3-phosphate dehydrogenase activity in incubation mixtures maintained under air, carbon monoxide and nitrogen. When 0.05 *M* dithiothreitol was added to the system, the differential effect of aerobic versus anaerobic incubation was abolished.

was measured under atmospheric conditions at varying concentrations of phosphate, and the enzyme was found to be proportionately more active at higher phosphate concentrations (Table).

When G-3-PD activity was measured under air, carbon monoxide and nitrogen, the enzyme was found to be nearly 300% more active under carbon monoxide and nitrogen. In the presence of 0.05 *M* dithiothreitol, G-3-PD activity was equivalent under aerobic and anaerobic conditions (Figure 2). Similarly, 2, 3-DPG synthesis was equivalent under aerobic and anaerobic atmospheres when 0.05 *M* dithiothreitol was added to the incubation system. At a concentration of 7 m*M* phosphate, 147 and 154 nmoles/ml of 2, 3-DPG were synthesized after 2 h under air and nitrogen, respectively.

G-3-PD is composed of 4 polypeptide subunits, each contributing 1 sulphydryl residue (cysteine 148) to the active center of the enzyme[11]. Oxidation of these sulphydryl residues is accompanied by inactivation of the enzyme. Recently peroxide has been shown to inactivate G-3-PD, presumably by the oxidation of the essential sulphydryl groups to sulphuric acid[12]. Enzyme inactivated in this manner may be reactivated by sulphydryl reagents.

The most likely explanation for the effect of anaerobic conditions upon G-3-PD activity reported in the current study would appear to be in the prevention of sulphydryl oxidation and subsequent inactivation of the enzyme. The effect of dithiothreitol would then derive both from the protection afforded sulphydryl residues and the reactivation of previously inactivated enzyme.

The accelerated rate of 2, 3-DPG synthesis in an anaerobic atmosphere in the absence of hemoglobin would therefore seem to be dependent upon increased G-3-PD activity. Experiments employing artificial 2, 3-DPG generating systems should guard against G-3-PD inactivation. What role this finding may play in the intact human erythrocyte remains to be demonstrated. With its high concentration of hemoglobin and reduced glutathione, the intact erythrocyte, under normal conditions, should not be vulnerable to G-3-PD inactivation.

It is of interest that when adult erythrocytes are incubated under nitrogen, 2, 3-DPG levels increase[13]. This increase has been ascribed to relief of 2, 3-DPG mutase from its product inhibition by 2, 3-DPG as a consequence of its binding to deoxyhemoglobin[5, 14]. The studies reported herein suggest that in an anaerobic atmosphere, at least in vitro, increased 1, 3-DPG synthesis secondary to augmented G-3-PD activity may also contribute to increased 2, 3-DPG synthesis[15].

Zusammenfassung. In Erythrozyten ist die Synthese von 2, 3-Diphosphoglycerat aus Fructose-1, 6-diphosphat in anaerobem Milieu um 18% höher als unter aeroben Bedingungen. Das anaerobe Milieu steigert die Aktivität der Glyceraldehyd-3-phosphat-dehydrogenase um 300%.

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Autoradiographic Identification of Rabbit Retinal Neurons that take up GABA

GABA (γ-aminobutyric acid) is now widely believed to be one of the mammalian neurotransmitters. It has recently been shown that the substance is actively taken up and retained by the central nervous tissue[1, 2], the mechanism somewhat resembling that for catecholamines in adrenergic neurons. The uptake of exogenous GABA into what might be presumed to be GABA neurons makes it possible to demonstrate these neurons autoradiographically, provided that diffusion can be controlled. The retina was chosen as test tissue because its regular structure permits simple classification of cells and

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