Oxygen-Hemoglobulin Dissociation Curves: Effect of Inherited Enzyme Defects of the Red Cell

Abstract. *The blood of a patient with a deficiency of hexokinase in the red cells and a decreased concentration of 2,3-diphosphoglycerate in the red cells showed an increased affinity for oxygen, whereas a patient with a deficiency of pyruvate kinase and an elevated concentration of 2,3-diphosphoglycerate in the red cells had blood with a decreased affinity for oxygen. Defects in red cell glycolysis may alter the oxygen affinity of blood by virtue of their effect on 2,3-diphosphoglycerate concentrations in red cells.*

Both 2,3-diphosphoglycerate and adenosine triphosphate (ATP) combine reversibly with adult deoxyhemoglobin, decreasing the affinity of the hemoglobin for oxygen and consequently shifting the oxygen-hemoglobin dissociation curve to the right (*1*). Because 2,3-diphosphoglycerate is quantitatively the most important organic phosphate in human erythrocytes, being present in approximately four times the concentration of ATP, attention has focused primarily on its role in altering the oxygen dissociation curve. It has been demonstrated in vitro that the binding of 2,3-diphosphoglycerate by deoxyhemoglobin A results in the relief of inhibition of 2,3-diphosphoglycerate mutase (E.C. 2.7.5.4) caused by 2,3-diphosphoglycerate (*2*). An increased synthesis of 2,3-diphosphoglycerate then occurs. Presumably the same effect is operative in vivo in the presence of hypoxemia. At high altitudes the concentration of 2,3-diphosphoglycerate is increased and the oxygen-hemoglobin dissociation curve is shifted to the right (*3*). Elevations in the concentration of 2,3-diphosphoglycerate in the red cells are also observed in adults with long-term hypoxemia as a consequence of lung disease or congenital heart disease (*4*). In this report the oxygen-hemoglobin dissociation curve of blood has been found to be altered in two patients with inherited defects of red cell metabolism.

The oxygen-hemoglobin dissociation curve and the concentration of 2,3-diphosphoglycerate in the red cells were determined in a subject with a deficiency of hexokinase in the red cells (*5*) and in a patient with a deficiency of pyruvate kinase. Heparinized blood (2 ml) from each subject was immediately placed in 4 ml of chilled 2*N* perchloric acid, homogenized, and extracted. The precipitate was extracted again with 0.5*N* perchloric acid, and the combined supernatants were neutralized with 5*N* KOH in an ice bath and stored overnight at 4°C. The samples were then centrifuged and the precipitate washed with 1 ml of water. The combined neutral extract was used for determination of the concentration of 2,3-diphosphoglycerate by the Schroter and Heyden modification of Krimsky's method (*6*). Blood (20 ml) was used for the determination of the oxygen-hemoglobin dissociation curve. Oxygen tensions were measured with the oxygen electrode (*7*) of a gas analyzer (IL model 113-SI), and oxygen saturations were measured spectrophotometrically (*8*). Eleven nonsmoking normal adults served as controls for the determination of the oxygen-hemoglobin dissociation curve, while an additional 20 normal subjects were used for determining the concentration of 2,3-diphosphoglycerate in the red cells.

The subject with deficiency of pyruvate kinase had a marked increase in the concentration of 2,3-diphosphoglycerate in the red cells and a decrease in the oxygen affinity of whole blood (Table 1 and Fig. 1). In this subject the concentration of 2,3-diphosphoglycerate in the red cells was 10,270 nmole per milliliter of red blood cells (normal, 4082 ± 515 nmole per milliliter of red blood cells), and the whole blood oxygen tension at 50 percent oxygen saturation (P_{50}) was 38 mm-Hg (normal, 24.4 ± 0.87). In contrast, the red cells of a patient with hexokinase deficiency had a concentration of 2,3-diphosphoglycerate of 2740 nmole per milliliter of red blood cells and a P_{50} value of 19 mm-Hg. From these data a regression line with a slope of 407 ± 45 nmole of 2,3-diphosphoglycerate per millimeter of Hg of oxygen tension was derived.

Anemia alone has been found to influence the concentration of the 2,3-diphosphoglycerate in red cells; anemic patients have an elevation in the concentration of 2,3-diphosphoglycerate in their red cells (*9*). We found two patients with similar degrees of anemia and reticulocytosis to have profoundly different oxygen-hemoglobin dissociation curves. In the patient with hexokinase deficiency the concentration of 2,3-diphosphoglycerate in red cells was reduced as a consequence of a block in glycolysis at the initial step of glucose phosphorylation. In contrast, the subject with a deficiency of pyruvate kinase had an elevation in 2,3-diphosphoglycerate concentration, as well as an accumulation of other glycolytic intermediates, above the metabolic defect in glycolysis. Mourdjinis and associates

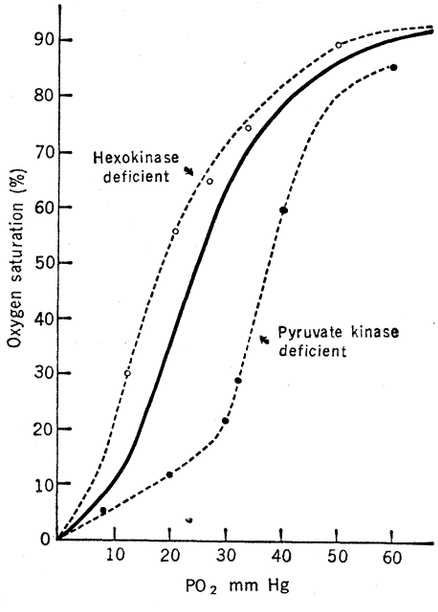

Fig. 1. The oxygen-hemoglobin dissociation curve as experimentally determined in normal subjects and in patients with hexokinase deficiency or pyruvate kinase deficiency (*p*H 7.346, 37°C).

Table 1. Concentration of 2,3-diphosphoglycerate (2,3-DPG) in the red cells, blood oxygen tension (mm-Hg) at 50 percent oxygen saturation (P_{50}), and hematologic findings.

Subject	2,3-DPG (nmole/ml of red blood cells)	P_{50} (mm-Hg)	Hemoglobin (gram percent)	Reticulocytes (percent)
Normals	4,082 ± 515	24.4 ± 0.87	15.8 ± 0.7	0.9 ± 0.2
Pyruvate kinase deficiency	10,270	38	10.7	22.5
Hexokinase deficiency	2,740	19	10.1	17.9

(*10*) reported an increase in the P_{50} in two subjects with pyruvate kinase deficiency and speculated that this increase could be a consequence of either the elevated concentrations of 2,3-diphosphoglycerate or the presence of a young red cell population. The finding of a disparity in oxygen-hemoglobin dissociation curves in two patients with similar degrees of anemia and reticulocytosis suggests that the concentration of 2,3-diphosphoglycerate is the factor responsible. These findings indicate that not only does the blood oxygenation affect red cell metabolism by altering the concentration of the 2,3-diphosphoglycerate in the red cells but also that intrinsic defects of red cell glycolysis can influence the affinity of hemoglobin for oxygen.

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Neuroglia Electrically Coupled to Neurons

Abstract. *Applied electric current is transmitted between mammalian glial cells grown in tissue culture. A similar electrical coupling exists between certain neurons as well as between neuroglia and neurons. Although this phenomenon may be a peculiarity of mammalian neural cells maintained in culture, it may, on the other hand, represent a phenomenon with greater neurophysiological significance, a process whereby neurons can become silent.*

Electrical coupling occurs between cells of tissue from various sources (*1*). Kuffler and his colleagues have described low resistance pathways that link glial cells in the nervous system of the leech (*2*) and of amphibians (*3*). It would be of interest to know whether similar connections occur between mammalian glial cells in order to establish a general characteristic of the cells, as well as to generate investigations of metabolic transport over what may be specialized membrane structures linking these cells together.

Since records from neighboring glial cells cannot be taken under visual control in the mammal, cultures of nervous tissue containing neurons and glia were chosen, especially since such cultures have been used successfully in the past for similar experiments that led to wider inquiries into the properties of glial cells in the intact brain (*4*). Explants were taken from the midbrain and cerebellum of rats 72 hours old and maintained in a tissue culture. Mammalian neural tissue that was grown on "flying cover slips" spread out into a thin layer of cells in which neurons and neuroglia became visibly distinct in 3 to 6 weeks. At a magnification of × 500 with phase optics, and with careful focusing, cell types and structures could be clearly defined in areas two or three cell layers thick (Fig. 1).

Under these conditions, glass microelectrodes filled with 3*M* KCl, 1.0*M* NaCl, or 1.0*M* potassium citrate could be easily manipulated into neurons and neuroglia, and the resting membrane potential (RMP) could be recorded. A technique similar to that used in the past (*5*) for measuring membrane resistance was used to determine electrical coupling of cells. A recording microelectrode was placed in one glial cell and a current-passing microelectrode in a second glial cell at least 250 μm from the cell bearing the recording electrode. We measured the voltage changes recorded in the first cell, which were produced by passing current into the second cell. The current electrode was then brought closer to the first cell by sampling successive cells until finally both electrodes were in the same cell. With this technique, a standard pulse of 5×10^{-8} amp for a 5-msec duration produced measurable voltage changes at distances up to approximately 200

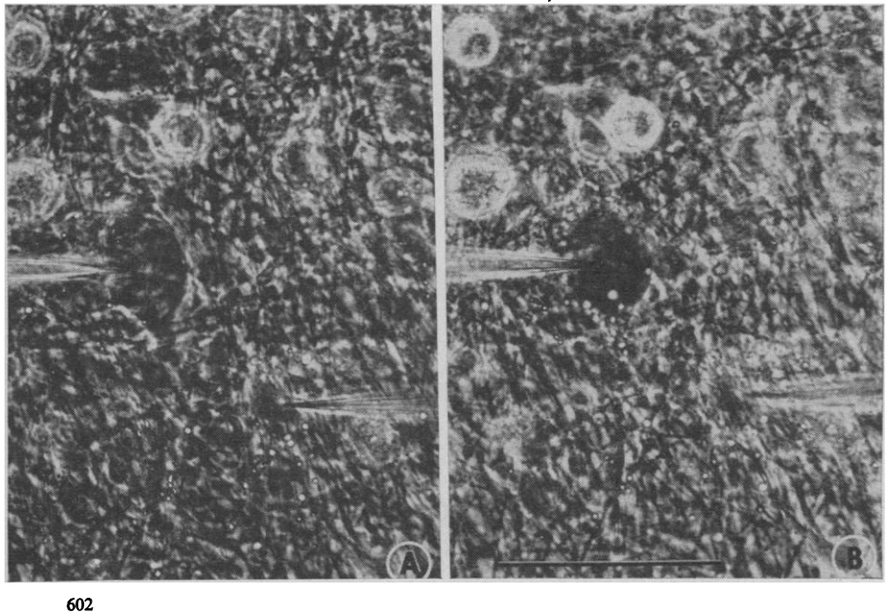

Fig. 1. (A) Phase photomicrograph illustrating placement of microelectrodes into the living cells of an explant being studied. The larger cell is a neuron and the smaller one is a neuroglia from a culture 28 days old. (B) Same field as in (A). The neuron has been stained blue (appearing dark within phase-contrast photomicrograph) after methyl blue was delivered electrophoretically into the cytoplasm. Bar is 50 μm.