CC/NUMBER 32
AUGUST 6, 1979

This Week's Citation Classic

Benesch R & Benesch R E. The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin.
Biochem. Biophys. Res. Commun. **26**:162-7, 1967.
[Columbia University, College of Physicians and Surgeons, Department of Biochemistry, New York, NY]

2,3 Diphosphoglycerate (DPG) was found to be a powerful allosteric effector of oxygen binding by hemoglobin. The dramatic decrease in oxygen affinity in the presence of this compound suggested its role in regulating oxygen release under physiological conditions. [The *SCI*® indicates that this paper has been cited over 585 times since 1967.]

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May 9, 1979

"Our discovery in 1966 of the effect of DPG on hemoglobin was quite surprising, since so much of the relevant information had been around for a long time. The compound had been isolated in large quantities from red cells by Greenwald in 1925.[1] During the next 40 years biochemists vainly searched for a metabolic function and its possible relation to oxygen transport was ignored, although it was suspected in some quarters, e.g., by Barcroft in 1921, that there might be 'some third substance present...which forms an integral part of the oxygen-hemoglobin complex.'[2]

"We were led to DPG by the casual question of a graduate student, Peter Model, who is now a professor at Rockefeller. He wanted to know why we always measured oxygen equilibrium curves in phosphate buffer and how much inorganic phosphate there was in human erythrocytes. A standard textbook of biochemistry showed right away that while there was precious little inorganic phosphate, the organic phosphate, 2,3 DPG, loomed very large, i.e., in almost equimolar amounts with hemoglobin. Since the compound was even commercially available, two and two were quickly put together. It became obvious that DPG combines preferentially with the deoxy form of hemoglobin. thereby lowering the oxygen affinity and facilitating the unloading of oxygen. It does this by prevailing on the hemoglobin molecule to return more readily to the state in which it was before it picked up oxygen.

"The interaction of DPG with hemoglobin quickly became a 'growth industry' since it had important implications in so many fields. The finding that only one DPG molecule acted allosterically on four oxygen finding hemes introduced a novel element into classical symmetry-dominated allosteric theory. It also added another dimension to the multibly-linked interactions of hemoglobin with ligands, e.g., the competition of CO_2 and DPG for the same site.

"On the physiological side quite a few loose ends were tied up. The much lower oxygen affinity of avian blood could be accounted for by the replacement of DPG by more powerful cofactors, i.e., inositol polyphosphates. In fact, the chicken switches from DPG to inositol pentaphosphate after hatching to cope with the altered oxygen requirement. In man, on the other hand, the higher oxygen affinity of fetal as compared with maternal blood is due to the lower affinity of fetal hemoglobin for DPG.

"A practical application of the DPG story was the realization that the increased oxygen affinity of blood during storage is due to destruction of DPG. As a result new blood storage media have been introduced. Perhaps the most striking physiological lesson has been the response of the red cell DPG level to oxygen need. Thus, ascent to high altitude rapidly raises DPG levels to 50% above normal and a similar increase is seen in a variety of diseases where too little oxygen reaches the tissues. In many anemias, such as sickle cell disease, patients with very low hemoglobin levels can often deliver normal amounts of oxygen because of their increased DPG concentration.

"It has been very satisfying to see how a relatively simple experiment has made it possible to find answers to so many old puzzles."

1. **Greenwald I.** A new type of phosphoric acid compound isolated from blood, with some remarks on the effect of substitution on the rotation of λ-glyceric acid. *J. Biol. Chem.* **63**:339-49, 1925.
2. **Adair G S, Barcroft J & Bock A V.** The identity of haemoglobin in human beings. *J. Physiol.* **55**:332-8, 1921.