Effect of Normal Metabolites on the Oxygen–Hemoglobin Equilibrium[1] (35058)

N. V. PANIKER AND E. BEUTLER

Division of Medicine, City of Hope Medical Center, Duarte, California 91010

The effect of 2,3-diphosphoglycerate (2, 3-DPG) and certain other organic and inorganic phosphates on the oxygen dissociation curve has been studied by different groups of workers in recent years (1–6). The right-shift of the oxygen dissociation curve in patients with anemia and in hypoxia (7) can now be accounted for by the increase in 2, 3-DPG levels of those erythrocytes. Though it is advantageous to have a shift in the oxygen dissociation curve to the right in anemia, making more oxygen available to the tissues at a given pO_2, such a property, unless properly regulated, can also have disadvantageous effects, through the lowering of the oxygen affinity of the blood in the alveoli. It is characteristic of many biological systems to be subjected to positive and negative control mechanisms. We have, therefore, considered the possibility that the effect of 2, 3-DPG might be modulated by some other substance within the erythrocyte, and have tested a variety of compounds (most of which are found as normal constituents of the red cell), singly and in combination with 2,3-DPG.

Materials and Methods. Blood which had been stored in ACD at 4° for 20–30 days was centrifuged at 1000*g* for 10 min. The plasma and buffy coat were aspirated and the cells were washed twice in 3 vol of cold isotonic phosphate–saline (9 vol of 0.9% NaCl + 1 vol of 0.1 *M* potassium phosphate buffer, pH 7.4). The washed red cells were hemolyzed by mixing with an equal volume of water and freezing and thawing once in a Dry Ice–acetone mixture. The hemolysate was centrifuged at 5000*g* for 10 min and the supernatant was dialyzed against 0.9% NaCl for 24 hr at 4°. The stock hemoglobin solutions were found to be stable for at least 1 week when stored under N_2 after deoxygenation in a frozen state. The concentration of hemoglobin adjusted to 5 g/100 ml by dilution with water, incorporating the compounds to be tested in a concentration of 3 m*M* (2 m*M* for pyridoxal phosphate) and potassium phosphate buffer to give a final concentration of 10 m*M*, pH 7.4, during the dilution process. The 2,3-DPG levels of the hemoglobin solutions were estimated as described previously (8). Measurement of oxygen–hemoglobin equilibrium was carried out by the mixing technique described by Edwards and Martin (9).

Results. The hemoglobin solutions prepared by the method described were found to be essentially free of 2,3-DPG. The effect of different concentrations of 2,3-DPG and of pyridoxal phosphate on the oxygen dissociation curve is shown in Fig. 1. In agreement with the observations of previous workers (2, 6, 10) there is a progressive shift of the O_2 dissociation curve to the right as shown by the increase in P_{50} as the 2,3-DPG or pyridoxal phosphate levels are raised. Beyond a concentration of 5 m*M* 2,3-DPG the increase in P_{50} is rather small and at about 2 m*M* final concentration, about half the maximum effect was observed. This concentration of 2,3-DPG is that found in the intact red cell and hence was chosen for subsequent studies in combination with other components. The maximum effect of pyridoxal was somewhat less than that of 2,3-DPG and was achieved at a somewhat lower concentration.

The P_{50} values of hemoglobin solutions

[1] This work was supported in part by Grant No. HE 07449 from the National Heart Institute, NIH.

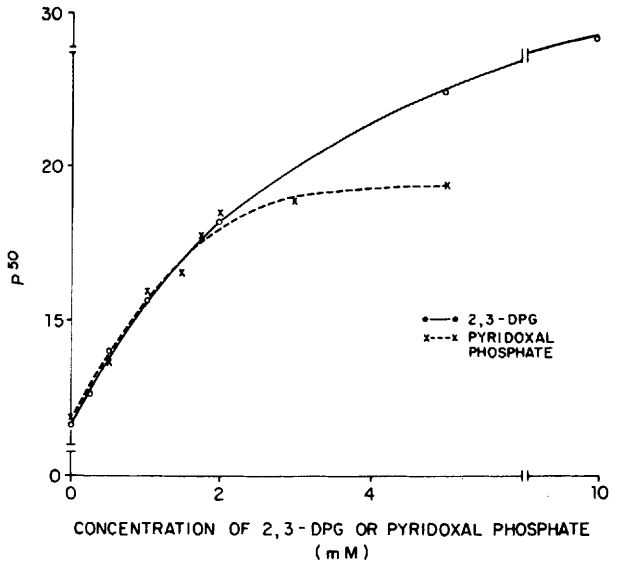

FIG. 1. The effect of 2,3-diphosphoglycerate (2,3-DPG) and pyridoxal phosphate on the oxygen dissociation curve of hemoglobin. The P_{50} value is the partial pressure of O_2 which one-half saturates hemoglobin at pH 7.4.

with a number of compounds (most of which are normal constituents of the red cell and are intermediates in the Embden Meyerhoff pathway of glucose metabolism) are shown in Table I. With all the compounds tested there was no difference in the P_{50}, whether the compound was added before the addition of 2,3-DPG or afterwards. Except for pyridoxal phosphate ond ATP, none of the compounds tested seem to modify the influence of 2,3-DPG on the oxygen dissociation curve either in the positive or negative direction. Since ATP and pyridoxal phosphate themselves are known to shift the O_2 dissociation curve of hemoglobin to the right, the observed change in presence of these compounds may be attributed to an additive effect.

Discussion. The present investigation fails to identify any substance besides ATP and pyridoxal phosphate which influences the O_2 dissociation curve, either in the presence of 2,3-DPG or its absence. It is of interset that red cell creatine, a compound whose function is unknown (11) and the concentration of which is reported to be correlated with that of 2,3-DPG (12) also has no effect. Many of the compounds employed in this study are known to bind with Hb (13) forming complexes with different electrophoretic mobilities. However, even structural analogues like 2-PGS and 3-PGA at concentrations higher than the concentration of 2, 3-DPG employed could not displace it from the Hb. It has been suggested (4) that the negatively charged phosphate ester is held in the central cavity of the diad axis of symmetry by electrostatic interaction. In oxyhemoglobin the central cavity is too small to admit 2,3-DPG and the conformational change which occurs on deoxygenation widens the cavity considerably (4, 14). It is of interest to note than even structurally related negatively charged phosphate esters like 2-PGA and 3-PGA do not appear to influence the 2, 3-DPG-binding site in the deoxygenated hemoglobin.

The fact that when these compounds were employed in the absence of 2,3-DPG the P_{50} remained unaltered clearly points to the high degree of specificity of the reaction between hemoglobin and 2,3-DPG.

Summary. A number of substances, most of them normal intermediates of red cell glucose metabolism, were tested for their effect on the shift in the oxygen dissociation curve

TABLE I. The Effect of Various Compounds on the P_{50} of Hemoglobin.[a]

Additions	No. of expts.	P_{50} In the absence of 2,3-DPG	P_{50} In presence of 2 m*M* 2,3-DPG
Nil	4	11.48 ± 0.26	18.37 ± 0.55
Glucose-6-phosphate	2	11.53	18.20
Fructose-1,6-diphosphate	2	11.75	18.41
Glyceraldehyde-3-phosphate	2	11.45	18.41
3-Phosphoglyceric acid	2	11.89	18.20
2-Phosphoglyceric acid	2	11.33	19.05
Phosphoenol pyruvic acid	2	11.26	17.99
Pyruvic acid	2	11.39	17.78
Lactic acid	2	11.82	17.99
Mannose-6-phosphate	2	11.46	18.20
Histidine	2	11.61	18.84
Sodium bicarbonate	2	11.20	18.41
ATP	2	17.65	26.00
PRPP	2	11.11	18.84
GSSG	2	11.89	19.5
Ribose-5-phosphate	2	10.96	17.99
β-Glycerophosphate	2	11.35	18.20
Creatine	2	11.71	18.62
Pyridoxal PO_4 (2 m*M*)	1	18.41	23.17

[a] All substances were tested at a concentration of 3 m*M*, except for pyridoxal phosphate, which was tested at 2 m*M*.

produced by 2,3-diphosphoglycerate. None of the substances tested, with the exception of ATP and pyridoxal phosphate had any effect on the dissociation curve, either in the presence or absence of 2,3-DPG. ATP and pyridoxal phosphate caused a rightward displacement of the dissociation curve, but this occurred both in the presence and absence of 2,3-DPG and was considered to be simply an additive effect.

1. Benesch, R., and Benesch, R. E., Biochem. Biophys. Res. Commun. **26**, 162 (1967).

2. Chanutin, A., and Curnish, R. R., Arch. Biochem. Biophys. **121**, 96 (1967).

3. Benesch, R., Benesch, R. E., and Enoki, Y., Proc. Nat. Acad. Sci. U.S.A. **61**, 1102 (1968).

4. de Verdier, C. H., and Garby, L., Scand. J. Clin. Lab. Invest. **23**, 149 (1969).

5. Lenfant, C., Torrance, J., English, E., Finch, C. A., Reynafarje, C., Ramos, J., and Faura, J., J. Clin. Invest. **47**, 2652 (1968).

6. Renthal, R., Benesch, R. E., Benesch, R., and Bray, B. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **29**, 732 (1970).

7. Rodman, T., Close, H. P., and Purcell, M. K., Ann. Intern. Med. **52**, 295 (1960).

8. Beutler, E., Meul, A., and Wood, L. A., Transfusion **9**, 109 (1969).

9. Edwards, M. J., and Martin, R. J., J. Appl. Physiol. **21**, 1898 (1966).

10. Charache, S., Grisolia, S., Fiedler, A. J., and Hellegers, A. E., J. Clin. Invest. **49**, 806 (1970).

11. Beutler, E., N. Engl. J. Med. **282**, 979 (1970).

12. Valeri, C. R., and Fortier, N. L., N. Engl. J. Med. **281**, 1452 (1969).

13. Chanutin, A., and Hermann. E., Arch. Biochem. Biophys. **131**, 180 (1969).

14. Muirhead, H., Cox, J. M., Mazzarella, L., and Perutz, M. F., J. Mol. Biol. **28**, 117 (1967).

Received June 12, 1970. P.S.E.B.M., 1970, Vol. 135.