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STUDIES OF OXYGEN DISSOCIATION IN RED CELLS WITH ABNORMAL HAEMOGLOBINS AND SOME ENZYME DEFICIENCIES

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Abstract—The oxygen dissociation properties in red cells in various congenital haemolytic states due to abnormal haemoglobins and enzyme deficiencies are discussed in relation to the degree of anaemia seen in these conditions. The results show that the main factor controlling the peripheral blood PCV is the erythropoietic response of the marrow to oxygen lack rather than the rate of haemolysis. This is because there is a high correlation between the oxygen affinity and the PCV.

ONE OF the puzzling features of chronic haemolytic diseases has been the paradox that in some patients mild haemolysis has resulted in severe anaemia, while in other patients with more severe haemolysis normal haemoglobin levels have been found. A possible explanation for this would be that the erythropoietin mediated marrow drive was controlled, not by the haemoglobin level *per se*, but by the oxygen delivery capacity of the blood. It is well known that changes in oxygen affinity of the blood alter the amount of oxygen that can be given off from the red cells on their passage through the tissues.

As the above paradox is well shown by the inherited haemolytic anaemias due to abnormal haemoglobins and enzyme deficiencies, we have studied the oxygen affinity of the red cells in these conditions. In order to do this, we devised a simple spectrophotometric method for determining the oxygen affinity of haemoglobin in red cells suspended in isotonic phosphate buffer.[1] This depends on the use of the second sample position of the Unicam SP 800 automatic recording spectrophotometer which minimizes the loss of spectral resolution due to light scattering. In Fig. 1, the oxygen affinity determined by us is plotted against the packed cell volume found in the patients studied. It can be seen that there is a high degree of correlation ($r = -0{\cdot}95$) between oxygen affinity and the degree of compensation by the bone marrow, as measured by the PCV. No such correlation was found when the time taken for 50 per cent survival ($t_{\frac{1}{2}}$) of ^{51}Cr labelled red cells in patients with similar diseases (collected from the literature) was compared with their PCV or haemoglobin levels. These results, therefore, indicate that an important factor controlling the level of red cell production is the oxygen affinity of the intracellular haemoglobin and imply that in chronic haemolysis the patients with a low haemoglobin and low oxygen affinity are, from the physiological point of view, no worse off than those with a high haemoglobin and high oxygen affinity.

The availability of a detailed structural model of the haemoglobin molecule and the knowledge of the amino acid substitution of the abnormal haemoglobins allows us to correlate the changes in oxygen dissociation with the molecular difference. The results of studies by a number of investigators show that there is little correlation between

the position and type of amino acid substitution and the change in oxygen affinity. It has been found, however, that substitutions at, or affecting one of the contacts between the α- and β-chains ($\alpha^1\beta^2$ contact) cause a gross reduction in haem–haem interactions. This correlates well with the finding that both the dissociation of the molecule into dimers ($\alpha_2\beta_2 \leftrightarrows 2\alpha\beta$)[2] and the movement between $\alpha\beta$-dimers on oxygenation,[3] take place at this contact as both have been implicated in the haem–haem interactions. Only one abnormal haemoglobin (Hb-Hiroshima, β143 (H21) His → Asp)[4] has a grossly reduced Bohr effect, and it is of interest that recent work has suggested that the residue involved is concerned with this function in the normal molecule.

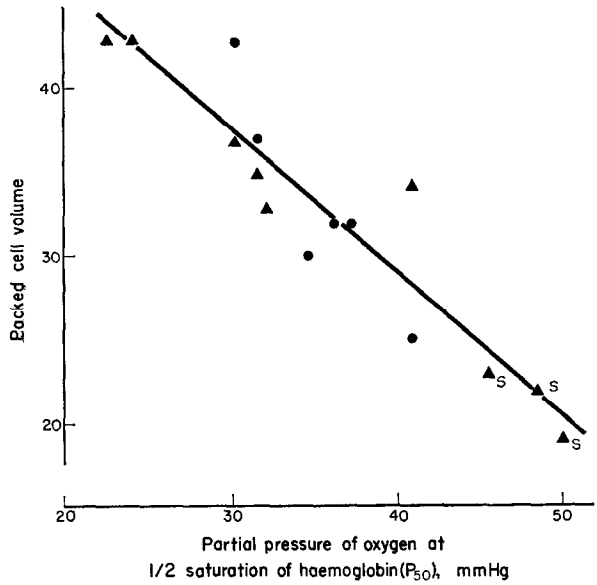

FIG. 1. Correlation of the oxygen affinity of red cells with the PCV of patients with chronic haemolytic anaemias in the steady state due to abnormal haemoglobins (▲), sickle-cell disease (S), and red-cell enzyme deficiencies (●). The oxygen affinity was determined on washed red cells suspended in an isotonic phosphate buffer, pH 7·1, at 37°.[1] The correlation coefficient, $r = 0{\cdot}95$, $P < 0{\cdot}001$. [Taken from E. R. HUEHNS and A. J. BELLINGHAM, *Br. J. Haemat.* **17**, 1 (1969).]

In 1967, it was shown by two groups of workers[5,6] that 2-3 diphosphoglycerate (2-3 DPG) which occurs in relatively high concentration in red cells compared with other cells, decreases the oxygen affinity of haemoglobin in solution, and this was due to increased binding of this compound by deoxyhaemoglobin. The results obtained by us on pyruvate kinase deficient red cells show that as expected these cells have a low oxygen affinity associated with a raised 2-3 DPG. On the other hand, in glucose 6-phosphate dehydrogenase (G6PD) deficient cells no change in oxygen affinity or 2-3 DPG was found. Other investigators [7] have shown that the well known decrease in oxygen affinity which occurs during the first 24 hr at high altitude is associated with increased levels of 2–3 DPG in the cells. These results indicate that 2–3 DPG is an important regulator of oxygen affinity of red cells, and it seems likely that it plays an

important role in the compensation for anaemia and blood loss. The exact way the level of 2-3 DPG is controlled in the cell is not clear. It is known that 2-3 DPG itself inhibits the formation of further 2-3 DPG and as it is more strongly bound to deoxy- than to oxy-haemoglobin the average proportion of reduced haemoglobin in the cell is probably an important factor regulating its synthesis; intracellular pH probably also plays a part. The discovery that 2-3 DPG regulates the oxygen affinity in the red cells has also explained the finding that, whereas foetal blood has a raised O_2 affinity, foetal red cell lysate has the same affinity as adult cell lysate. It is now known that Hb-F without 2-3 DPG has a lower oxygen affinity than Hb-A without 2-3 DPG. However, Hb-F binds the regulator molecule much less than does Hb-A[8] and, as a result, for an equivalent amount of intracellular 2-3 DPG, the oxygen affinity of red cells containing Hb-F is much higher than that of Hb-A containing cells.[9]

Thus, it can be seen that the study of the inherited abnormalities of red cell metabolism and of the abnormal haemoglobins has helped us in the understanding of the control of erythropoietic response in haemolytic anaemias and is also helping us to unravel the mechanism controlling the oxygen affinity in the cell.

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