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Effects of 2,3-Diphosphoglycerate and Other Organic Phosphate Compounds on Oxygen Affinity and Intracellular pH of Human Erythrocytes*

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Summary. The effects of changes of the 2,3-DPG content and of the total concentration of organic phosphates on the oxygen affinity and the intracellular pH of human erythrocytes were studied. The oxygen affinity as characterized by the P_{50} (oxygen tension at 50% O_2 saturation) increases from 15 to 45 mm Hg when the 2,3-DPG concentration is elevated from 0.1 to 24 μmoles/g by incubation of erythrocytes in the presence of inosine, pyruvate and phosphate.

In cells containing normal concentrations of 2,3-DPG, but accumulating high amounts of other organic phosphates during incubation with inosine and phosphate, the P_{50} was found to rise up to 36 mm Hg. This effect as well as a considerable part of the 2,3-DPG effect on the oxygen affinity of intact erythrocytes is due to a shift of the Donnan equilibrium induced by the accumulation of non-penetrating phosphate anions and consecutive changes of the intracellular pH, which in turn alter the oxygen affinity via the Bohr effect of hemoglobin.

The intracellular pH is related to the intracellular concentration of organic phosphates (extracellular pH 7.40) by the equation:

$$pH_i = 7.306 - 0.0083 \cdot P_{org} \text{ (μmoles organic P/g).}$$

This dependency agrees closely with the theoretical relationship between the intracellular pH and the concentration of organic phosphates calculated from the osmolarities and the net charges of non-penetrating cell constituents.

After correction of the oxygen affinities to a constant intracellular pH the P_{50} does not further increase in cells containing 2,3-DPG concentrations above 8 μmoles/g and remains unaltered in erythrocytes accumulating other organic phosphates.

Key-Words: Erythrocyte — Oxygen Affinity of Hemoglobin — 2,3-Diphosphoglycerate — Donnan Equilibrium — Intracellular pH.

Schlüsselwörter: Erythrocyt — Sauerstoff-Affinität von Hämoglobin — 2,3-Diphosphoglycerat — Donnan-Gleichgewicht — Intracellulärer pH.

* Part of the results was presented at the 38th Meeting of the German Physiological Society, Erlangen, Sept. 29 to Oct. 2, 1970 [12].

For a long time the oxygen affinity of red cells has been regarded as a parameter which is influenced in vivo only by changes of blood pH, of partial pressure of carbon dioxide and of the temperature [37]. A few years ago, however, Chanutin an Curnish [6] as well as Benesch and Benesch [3] could demonstrate that the oxygen affinity of hemoglobin is remarkably decreased by 2,3-DPG[1] and ATP, normal constituents of most of mammalian erythrocytes.

In human red blood cells the effect of 2,3-DPG is quantitatively the more important since its concentration exceeds about four times that of ATP. Moreover, 2,3-DPG levels of human erythrocytes are known to change considerably under various conditions. These concentration changes are paralleled by substantial shifts of the oxygen dissociation curve of human blood [7,16,25,31,39,40,42]. An intraerythrocytic response of this type is assumed to play an important role in improving the tolerance of men to different kinds of hypoxia [5,10,26,30].

In hemoglobin solutions the effect of 2,3-DPG on the oxygen affinity proved to be almost maximal at concentrations of 2,3-DPG normally found in human erythrocytes [3,36]. From this observation it was concluded that changes of the 2,3-DPG level within the physiological range should scarcely have any effect on the oxygen affinity of red blood cells [3]. In contrast, it was repeatedly observed that an elevation of the 2,3-DPG content of intact red blood cells above the normal value results in a further decrease of the oxygen affinity [7,25,31,42]. The present studies have been performed in order to investigate this discrepancy between the effects of 2,3-DPG on the oxygen affinities of hemoglobin solutions and of intact red blood cells.

Methods

Blood was drawn from non-smoking volunteers, heparin being used as anticoagulant (0.2 mg/ml blood). After centrifugation (4,500 *g*) plasma and buffy coat were removed and the red cells washed twice with an excess of Locke solution (composition cf. [19]). The cells were then incubated for 10—180 min at 37° C (pH 7.35—7.40, hematocrit 10—15%) in isotonic media containing inosine (10 mM), pyruvate (10 mM), orthophosphate (50 mM), and NaCl (75 mM) (= IPP media). In a special series of experiments pyruvate was replaced by NaCl (= IP media). Inosine and sodium pyruvate were obtained from E. Merck AG., Darmstadt.

For the incubation of erythrocytes without glucose the cells were washed three times in large volumes of glucose-free Locke solution at 22° C and resuspended in the same medium (final glucose concentration less than 0.05 mM, hematocrit 40%, 37° C, pH 7.35—7.40, incubation time: 4—8 h).

Abbreviations. 2,3-DPG: 2,3-Diphosphoglycerate; ATP: Adenosinetriphosphate; F-1,6-P: Fructose-1,6-diphosphate; DAP: Dihydroxyacetonephosphate; GA-3-P: Glyceraldehyde-3-phosphate; P_{org}: Total concentration of acid-soluble organic phosphates; IPP: Inosine-pyruvate-phosphate; IP: Inosine-phosphate; Subscripts $_i$ and $_e$: intracellular and extracellular; P_{50}: Oxygen tension at 50% oxygen saturation (mm Hg).

The pH was controlled using a glass electrode and adjusted, when necessary, by addition of 0.3 n NaOH and 0.3 n HCl. Aliquots of the red blood cells, washed three times with an excess of saline at 4°C, were either extracted by perchloric acid or used to determine the oxygen dissociation curve and the intracellular pH.

Oxyhemoglobin Dissociation Curves. Samples of fresh or incubated red blood cells were resuspended in their own plasma. 0.1 ml of the cell suspensions were equilibrated in a tonometer for 15 min with H_2O saturated gas mixtures containing CO_2 (40 mm Hg) and varying proportions of O_2 and N_2, P_{O_2} values ranging between 10 and 75 mm Hg (37°C, pH 7.35—7.45; Blood Micro System BMS 2, Radiometer Co., Copenhagen). The pH of each equilibrated sample was measured anaerobically by a glass microelectrode at 37°C. Oxygen saturation was determined spectrophotometrically (Zeiss, PMQ II) [38] and corrected to a pH of 7.40 using a Bohr factor of 0.48. For each blood sample five points were determined in duplicate within the range of 20—80% O_2 saturation in order to obtain the oxygen dissociation curve. The P_{50} values (oxygen tension at 50% oxygen saturation) were computed according to Hill's equation [23].

Intracellular pH. The interrelationship between the plasma pH and the intracellular pH of red blood cells containing varying amounts of organic phosphates was studied according to the methods described by Funder and Wieth [17]. Prior to the resuspension of fresh or incubated red cells the pH of the plasma was varied by addition of 0.15 n HCl in 0.15 n NaCl and of 0.15 n NaOH. 0.9% NaCl was added in amounts yielding a constant dilution of the plasma proteins by 33%. The red cell suspensions (hematocrit 30—40%, pH 6—9) were equilibrated with air and transferred to polyethylene tubes. The tubes were sealed and incubated in a water bath at 37°C for 20 min. After 5 min of centrifugation at 37°C (11,000 *g*) the packed red cells were lyzed by freezing (−20°C) and thawing. Measurements of the pH in the supernatant and in the lyzed red cells were carried out in triplicate (S.D. = 0.009 pH units) at 37°C using a microelectrode equipment (Radiometer Co., Copenhagen, calibration with Radiometer precision buffers).

Phosphate Compounds. From the perchloric acid extracts, which were neutralized by the addition of KOH, 2,3-DPG, ATP and inorganic phosphate were isolated by means of paper chromatography [20] and quantitated by phosphate determinations [18]. F-1,6-P and the triose phosphates (DAP + GA-3-P) were determined enzymatically [4]. The concentration of acid-soluble organic phosphates (P_{org}) was computed by subtracting the values of inorganic phosphate from those of the total acid-soluble phosphorus which was analyzed after complete acid hydrolysis of the extracts. All concentration values (μmoles/g or μmoles P/g erythrocytes) refer to red cells suspended at a pH of 7.35 to 7.40 and are not corrected to a constant dry weight.

Results and Discussion

I. Effect of the 2,3-DPG Concentration of Human Erythrocytes on the Oxygen Affinity of Blood

The 2,3-DPG concentration of human erythrocytes can be elevated in vitro to more than five times its normal value within short periods of time by incubating the cells in the presence of inosine, pyruvate and phosphate [9, 14, 29]. On the other hand the concentration of 2,3-DPG decreases rapidly in red cells incubated without any substrate [13]. By application of these two procedures, the 2,3-DPG content of human red blood cells was varied between 0.1 and 24 μmoles/g.

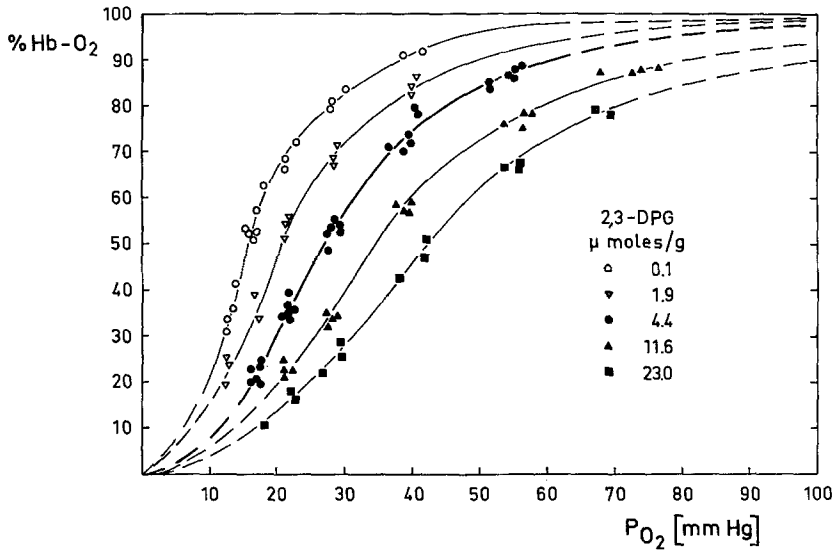

Fig. 1. Oxyhemoglobin dissociation curves of human erythrocytes containing different concentrations of 2,3-DPG (pH_e 7.40; 37° C; P_{CO_2} 40 mm Hg)

The displacements of the oxygen dissociation curve of human blood caused by such concentration changes of 2,3-DPG are demonstrated in Fig. 1. The oxygen tension at 50% O_2-saturation (P_{50}) decreases from 27 to 15 mm Hg when the concentration of 2,3-DPG is lowered from its normal level of 4.4 µmoles/g erythrocytes to a value of 0.1 µmoles/g. Elevation of the 2,3-DPG level to 23 µmoles/g leads to a remarkable right-hand shift of the oxygen dissociation curve (P_{50} = 43 mm Hg). These findings demonstrate the fundamental role of 2,3-DPG in determining the oxygen affinity of human blood

The changes of the oxygen saturation brought about by 2,3-DPG at high oxygen tensions (70—100 mm Hg) are comparatively small. In contrast, at a low oxygen tension of 30 mm Hg, for instance, the oxygen saturation decreases from 83 to 28% when the concentration of 2,3-DPG is raised from 0.1 to 23 µmoles/g red blood cells.

Obviously 2,3-DPG operates as a regulator of the oxygen affinity of human red blood cells mainly at the oxygen tensions which are found in capillaries where oxygen has to be released from hemoglobin to the tissues.

2,3-DPG did not significantly alter the heme-heme interaction of hemoglobin characterized by the Hill coefficient n [2.65 $\pm$ 0.08 (S.D.) in our experiments]. The effect of 2,3-DPG on the oxygen affinity of blood

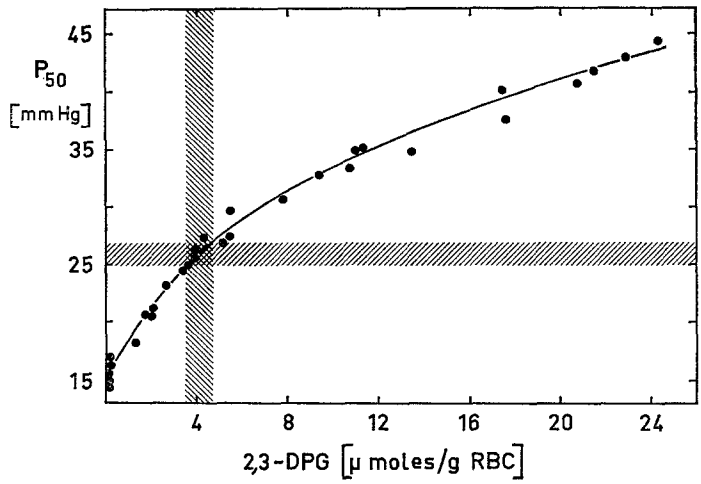

Fig. 2. Dependency of the P_{50} of human erythrocytes on the 2,3-DPG concentration (pH_e 7.40; 37°C; P_{CO_2} 40 mm Hg). Normal ranges indicated by hatched areas

can thus be described entirely by the changes of the P_{50} values. According to Fig. 2 a curvilinear relationship exists between the P_{50} and the concentration of 2,3-DPG in red cells. In the range between 0.1 and 6 μmoles of 2,3-DPG/g the curve corresponds closely to that found in hemoglobin solutions [3, 8, 36]. At 2,3-DPG levels exceeding 8 μmoles/g, however, a further and almost linear increase of the P_{50} is observed in intact erythrocytes, which does not occur in hemoglobin solutions.

II. Effect of 2,3-DPG and other Organic Phosphates on the Donnan Equilibrium of Human Red Cell Suspensions

Red cell suspensions—in contrast to hemoglobin solutions—consist of two compartments (red cells and medium), which are separated by a membrane impermeable to hemoglobin and 2,3-DPG, but permeable to small anions (Cl^-, HCO_3^-, OH^-). The distribution of penetrating ions between these two compartments follows Donnan's law [11] and can be described by the equation

$$\frac{Cl_i^-}{Cl_e^-} = \frac{HCO_{3_i}^-}{HCO_{3_e}^-} = \frac{OH_i^-}{OH_e^-} = \frac{H_e^+}{H_i^+} = r. \quad (1)$$

The ratio r is mainly determined by the intracellular concentration of non-penetrating charged molecules such as hemoglobin and the organic phosphate anions [11, 34, 41]. Changes of the 2,3-DPG concentration in intact red blood cells should therefore produce a shift of the Donnan

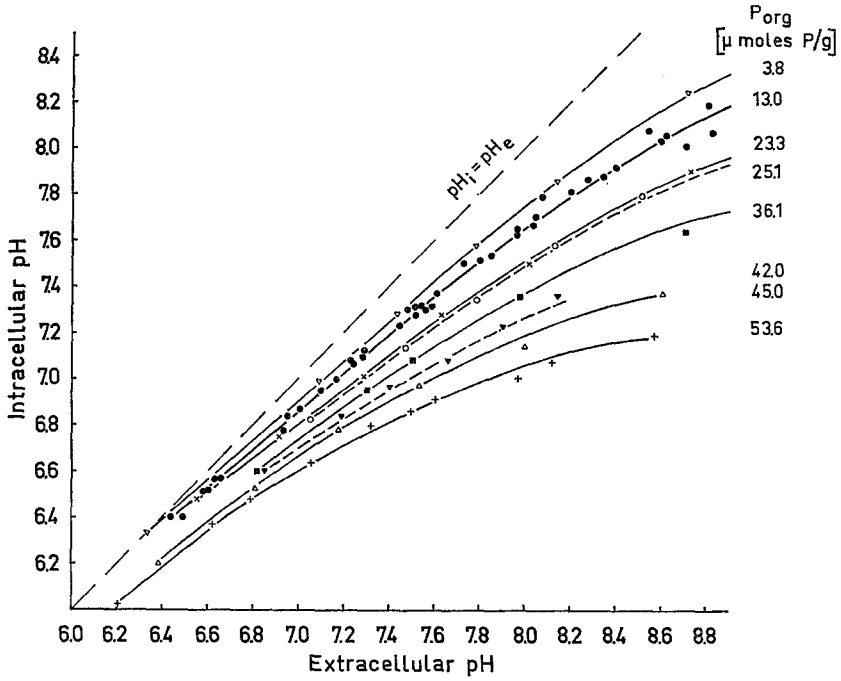

Fig. 3. Relation between the extracellular and the intracellular pH in human blood at different intracellular concentrations of organic phosphates. P_{org} was varied by incubation of the cells without substrate or in the presence of IPP (solid lines) and of IP (broken lines) (for further experimental details see Methods). The concentrations of the individual phosphate compounds are given in Table 1

equilibrium including changes of the intracellular pH. These changes of the intracellular pH, in turn, should be followed by changes of the oxygen affinity of hemoglobin and could thus be responsible for the differences observed between the oxygen affinities of intact red blood cells and of hemoglobin solutions at high concentrations of 2,3-DPG.

According to the theory of Donnan equilibria a decrease of the intracellular pH should not be a peculiarity of erythrocytes containing high amounts of 2,3-DPG, but rather a general consequence of the intracellular accumulation of any other non-penetrating organic phosphates.

a) Experimental Results

The validity of this assumption was tested in experiments, in which the relationship between the intracellular and the extracellular pH was measured in red cells containing different concentrations of 2,3-DPG or of other organic phosphates after various periods of incubation in IPP or IP media (for details see Methods). A series of curves was obtained correlating intracellular and extracellular pH values (Fig. 3). In agreement with

Table 1. *Concentrations of organic phosphates in human erythrocytes incubated without substrate or in the presence of either inosine, pyruvate and phosphate (IPP) or inosine and phosphate (IP)*[a]. *The intracellular pH values are given in Fig. 3*

Substrates	Time of incubation min	P_{org} µmoles P/g	2,3-DPG µmoles/g	F-1,6-P µmoles/g	ATP µmoles/g	DAP+GA-3-P µmoles/g	Unidentified organic P µmoles P/g	Dry weight %
Without substrate	420	3.8	0.8	< 0.1	0.2	< 0.1	1.4	33.1
Controls	0	13.0	3.8	< 0.1	1.2	< 0.1	1.6	34.1
IPP	20	23.3	7.7	0.5	1.3	0.6	2.3	35.6
IP	30	25.1	4.0	4.0	1.1	0.3	5.5	35.6
IPP	45	36.1	11.3	1.5	1.6	1.7	4.0	38.3
IP	180	42.0	4.7	8.7	1.3	1.2	10.4	37.9
IPP	90	45.0	16.1	1.6	1.5	1.7	3.2	39.3
IPP	180	53.6	20.7	0.8	1.5	1.4	3.8	38.7

[a] For methodical details cf. Methods.

Donnan's law the curves are shifted to more acid intracellular pH values at high concentrations of organic phosphates and to a more alkaline pH after diminution of the organophosphate concentration [22]. These effects on the intracellular pH become more pronounced with increasing extracellular pH values.

In addition to the measurements of the intracellular pH the main organic phosphate fractions were determined after incubation of the cells in IPP or IP media. The metabolite patterns differ considerably (Table 1). In IP treated cells the rise of the sum of organic phosphates is predominantly due to an increase of F-1,6-P [1], whereas in IPP treated cells it is mainly caused by the accumulation of 2,3-DPG.

From these data and those given in Fig. 3 it may be concluded that the intracellular pH decreases in the presence of high concentrations of both 2,3-DPG and F-1,6-P. The extent of the shift of the intracellular pH to more acid values seems to depend only on the total concentration of organic phosphates.

The question now arises whether these changes of the intracellular pH can be described quantitatively in terms of Donnan equilibria. In order to clarify this problem the experimental relationship between the

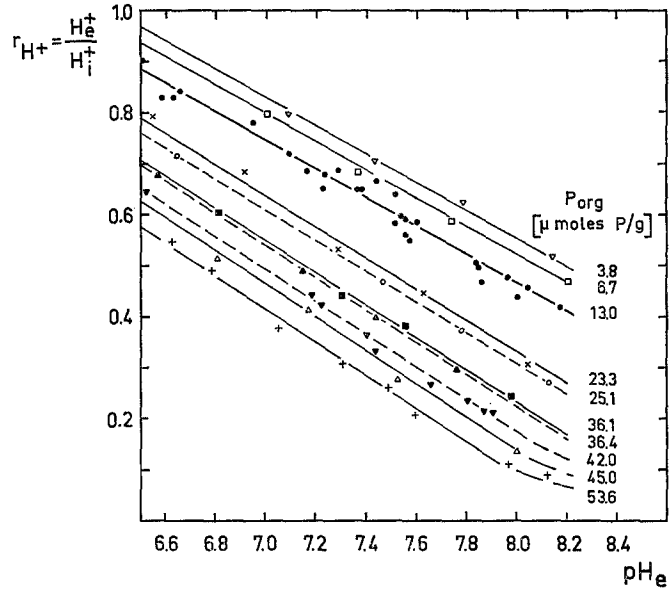

Fig. 4. Donnan distribution ratio r_{H^+} in human blood as function of the extracellular pH and of the intracellular concentration of organic phosphates

intracellular pH and the total concentration of organic phosphates has to be compared with the theoretical relationship between both parameters calculated from the Donnan theory.

The intracellular pH values to be related to the concentrations of organic phosphates have to be determined at identical extracellular pH values. In the experiments described above, however, the extracellular pH was varied by the addition of fixed amounts of HCl or NaOH. The extracellular pH values thus obtained were not identical in the different experiments (see Fig. 3), since changes of the concentrations of organic phosphates were accompanied by changes of the water content and therefore of the hemoglobin concentration and of the buffering capacity [9]. To overcome this difficulty the required intracellular pH values were calculated from the experimental data in the following way.

In a first step distribution ratios $r_{H^+} = H_e^+/H_i^+$ were calculated from the pH values given in Fig. 3. By plotting these r_{H^+} values against the extracellular pH, curves were obtained which are almost linear and parallel to each other up to an extracellular pH of 8.0 (Fig. 4). The distribution ratios of hydrogen ions can therefore be related to the extracellular pH by equations of the type

$$r_{H^+} = a - b \cdot pH_e, \tag{2}$$

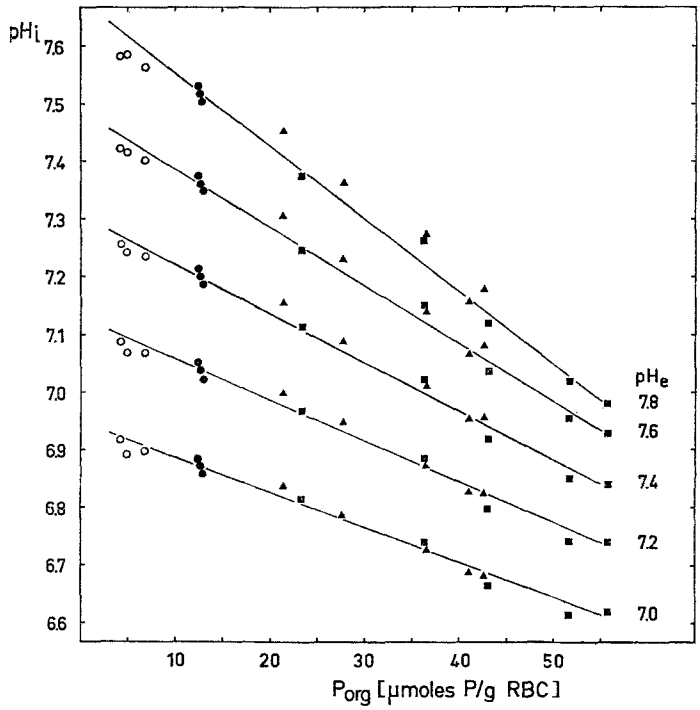

Fig. 5. Dependency of the intracellular pH of human erythrocytes on the concentration of organic phosphates at different extracellular pH values. The concentration of the organic phosphates was changes by incubation without substrate (○), in the presence of inosine and phosphate (▲), or of inosine, pyruvate and phosphate (■); • controls

where a and b are the constants characteristic for each value of P_{org}. In normal blood a and b were found to be 2.695 and 0.279, respectively. These values are in agreement with the results of other authors [17].

Equation [2] permits the determination of r_{H^+} for any extracellular pH value, provided a and b are known. By inserting these r_{H^+} values into the equation

$$pH_i = pH_e + \log r_{H^+} \tag{3}$$

derived from equation [1], the intracellular pH, which is related to a certain extracellular pH, can be calculated.

The intracellular pH values thus obtained for identical values of the extracellular pH are plotted against the organic phosphate concentration of the cells in Fig. 5. Obviously the intracellular pH decreases linearly with rising concentrations of organic phosphates, independent

of the molecular species of the individual phosphate compounds responsible for the concentration changes. The slope and the position of the regression lines varies with the extracellular pH. At pH 7.40, for instance, the relationship between the intracellular pH and the organic phosphate concentration can be described by the equation

$$pH_i = 7.306 - 0.0083 \times P_{org} \quad (\mu\text{moles P/g}). \tag{4}$$

Accordingly the intracellular pH decreases from 7.25 to 6.85 when the organic phosphate concentration is raised from 5 to 55 μmoles P/g.

b) Theoretical Considerations

In order to obtain the relationship between the concentration of organic phosphates and the intracellular pH resulting from a Donnan distribution of hydrogen ions, theoretical intracellular pH values were calculated from the measured concentrations of organic phosphates and hemoglobin by the following equation:

$$pH_i = pH_e + \log\left(1 - \frac{N_i + A_i^- - A_e^-}{2\,(B_e^+ - A_e^-)}\right) \tag{5}$$

N_i = Total osmolarity of intracellular non-penetrating compounds (mosmoles/l H_2O).

A_i^-, (e) = Total anion equivalency of intracellular (extracellular) non-penetrating compunds (meq/l H_2O).

B_e^+ = Total concentration of extracellular cations (meq/l H_2O).

This equation was derived by combining equation [3] with a modified version [9] of van Slyke's classical equation 10 which describes the influence of non-penetrating compounds on the distribution of penetrating ions in blood [41]. A_e^- and B_e^+ were taken to be 10 and 160 meq/l H_2O, respectively. N_i and A_i^- were calculated from the measured concentrations, the osmotic coefficients and the dissociation constants of the individual phosphate compounds and of hemoglobin [24, 27, 28, 33].

This procedure is illustrated for two examples in Table 2. In normal erythrocytes the major part of the anion equivalency of non-penetrating cell constituents originates from hemoglobin. The ratio of the anion equivalencies between hemoglobin and the organic phosphates amounts to 1.3. In cells containing high concentrations of organic phosphates (after 3 h of incubation in the presence of IPP) the contribution of hemoglobin to the total equivalency of non-penetrating intracellular anions becomes almost negligible and the ratio of the anion equivalencies decreases to about 0.1. Furthermore it becomes evident that the intracellular pH values calculated on the basis of equation [5] for the two examples agree closely with the intracellular pH values determined experimentally.

Table 2. *Concentrations and equivalencies of non-penetrating compounds within human red cells determining the Donnan distribution of penetrating ions*[a]. *Extracellular pH 7.40*

	Normal cells[b] ($n = 6$)	Cells incubated for 3 h with IPP
Osmolarity[c] (mosmoles/l H_2O)		
2,3-DPG	5.3 ± 0.4	31.6
ATP	1.7 ± 0.4	2.3
Unidentified organic P[d]	1.7 ± 0.4	5.3
Hemoglobin	29.5 ± 3.5	36.6
N_i	38.2 ± 4.0	75.8
Anion equivalency[e] (meq/l H_2O)		
2,3-DPG	20.6 ± 1.1	115.2
ATP	6.3 ± 0.7	8.4
Unidentified organic P	5.9 ± 1.4	18.0
Hemoglobin	41.8 ± 2.2	16.7
A_i^-	74.6 ± 4.2	158.3
Intracellular pH		
Experimental	7.195 ± 0.005	6.847
Calculated	7.218 ± 0.018	6.799

[a] For experimental details see Methods.

[b] Mean values ± S.D.

[c] Osmolarities were obtained by multiplying the molar concentrations by the the osmotic coefficients. The osmotic coefficients of the organic phosphates were assumed to be 1.0, those of hemoglobin were adopted from Fig. 3 of [29].

[d] The unidentified phosphates were assumed to consist of diphosphates.

[e] Anion equivalencies were obtained by multiplying the molar concentrations by the number of negative charges per mole at the measured intracellular pH. The charges of ATP, 2,3-DPG and hemoglobin were taken from the literature [24, 27, 33). The charge of the unidentified organic P was assumed to have a value of −1.7 meq/mole of P.

The calculated and the experimental intracellular pH values proved to be very similar in all other cases too. Therefore the relationship between the theoretical intracellular pH values and the total concentration of organic phosphates as described by the equation

$$pH_i = 7.323 - 0.0098 \cdot P_{org}\ (\mu\text{moles P/g}) \qquad (6)$$

turned out to differ only slightly from the relationship determined experimentally [equation (4)].

From this result it may be concluded that the considerable decrease of the intracellular pH in red cells accumulating large amounts of organic phosphates can be fully explained by a progressive shifting of the Donnan equilibrium of penetrating ions between cells and medium.

III. Effect of Donnan-Induced Changes of the Intracellular pH on the Oxygen Affinity of Blood

As an important consequence of the decrease of the intracellular pH the oxygen affinity of erythrocytes becomes diminished due to the Bohr effect of hemoglobin. Thus two effects are responsible for the changes of the oxygen affinity observed in intact erythrocytes containing high concentrations of 2,3-DPG:

1. The specific action of 2,3-DPG on the hemoglobin molecule and
2. the unspecific effect of changes of the intracellular pH, which has been recently demonstrated in sheep red cells too [2].

The relative contributions of the specific and the unspecific component to the changes of the oxygen affinity can be estimated when the measured P_{50} values are corrected to the normal intracellular pH of 7.20 by equation (4) and a Bohr factor of 0.48 (Fig. 6). The resulting "true" relationship between the 2,3-DPG concentration and the P_{50} of intact red blood cells (dotted line of Fig. 6) characterizes the specific effect of 2,3-DPG which is of particular importance in a concentration range up to 8 μmoles 2,3-DPG/g. The changes of the oxygen affinity in cells containing 2,3-DPG in higher concentrations is attributable almost entirely to the unspecific effect of intracellular pH changes.*

The curve of the specific component of the 2,3-DPG effect in intact erythrocytes coincides with that obtained for the influence of 2,3-DPG on the oxygen affinity of hemoglobin solutions [3] and undiluted hemolysates [36]. Thus the apparent discrepancies between the effects of 2,3-DPG in hemoglobin solutions and in intact human erythrocytes result only from the impermeability of the red cell membrane to 2,3-DPG and the consecutive changes of the intracellular pH.

The unspecific effect of organic phosphates on the oxygen affinity of intact erythrocytes can be directly demonstrated in cells accumulating organic phosphates other than 2,3-DPG which do not influence the oxygen affinity of hemoglobin solutions [32]. According to Fig. 7 the P_{50} of erythrocytes incubated in the presence of inosine and phosphate increases in proportion to the total concentration of organic phosphates (solid line) although the concentration of 2,3-DPG remains fairly constant. From the

* *Note added in proof.* As was recently demonstrated by Bellingham, Detter, and Lenfant (J. Clin. Invest. **50**, 700 (1971)] 35% of the change in the oxygen affinity of human blood resulting from *in vivo* alterations of red cell 2,3-DPG levels in the range between 9 and 19 μmoles/g hemoglobin (this corresponds to about 3.1 and 6.4 μmoles 2,3-DPG/g erythrocytes) are caused by the effect of 2,3-DPG on the red cell pH. This value fits well with our *in vitro* measurements. It shall be noted, however, that the Donnan-mediated pH effect is less important at lower 2,3-DPG levels, whereas the percentage of the change in the P_{50} caused by the pH effect of 2,3-DPG amounts to about 100% at 2,3-DPG concentrations above 7—8 μmoles/g erythrocytes.

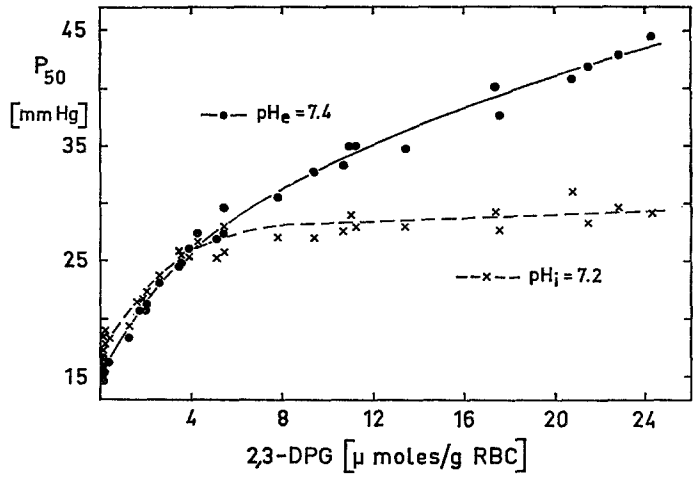

Fig. 6. Relationship between the 2,3-DPG concentration of human erythrocytes and the P_{50} of whole blood (37°C; P_{CO_2} 40 mm Hg). •——• P_{50} determined at an extracellular pH of 7.40 ×---× P_{50} corrected to an intracellular pH of 7.20

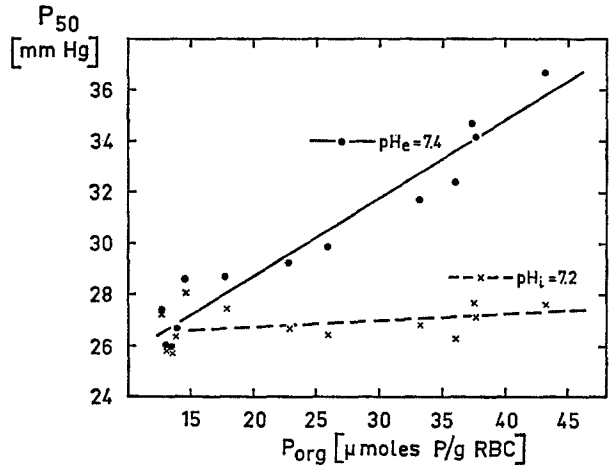

Fig. 7. Unspecific alterations of the P_{50} of human erythrocytes containing elevated amounts of organic phosphates after incubation in the presence of inosine and inorganic phosphate. The 2,3-DPG concentration was 4.5 ± 0.5 μmoles/g cells. •——• P_{50} determined at an extracellular pH of 7.40. ×---× P_{50} corrected to an intracellular pH of 7.20

P_{50} values corrected to a constant intracellular pH (broken line) it becomes evident that the changes of the oxygen affinity observed under these conditions are actually caused by the intracellular acidification.

The accumulation of non-penetrating organic phosphates and the resulting decrease of the intracellular pH are also responsible for a number of other phenomena previously observed in cells containing increased concentrations of 2,3-DPG: a) The decrease of the rate of glycolysis [9] which results most probably from a pH-induced inhibition of phosphofructokinase [33]; b) the shrinking of red cells [9] and the diminution of the intracellular concentration of chloride and bicarbonate [34] due to a shift of the Donnan equilibrium and c) the selflimitation of the adaptive increase of the 2,3-DPG concentration during hypoxia which is mainly caused by pH induced changes of the glycolytic metabolism [15].

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