Oxyhemoglobin Affinity in Chronic Pulmonary Granulomatosis (Sarcoidosis) and Fibrosis[1, 2]

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SUMMARY

Oxyhemoglobin affinity was evaluated in 21 patients with chronic arterial hypoxemia due to pulmonary fibrosis and granulomatosis (sarcoid) by measurements of the blood oxygen tension required to achieve 50 per cent oxyhemoglobin saturation at 37°C and pH 7.40 (P_{50}), erythrocytic 2,3-diphosphoglycerate (2,3-DPG) and adenosine triphosphate. Twelve normal persons served as control subjects. Mean (± SD) physiologic values characterizing these patients included: forced vital capacity, 67.8 ± 17.6 per cent of predicted ($P < 0.01$); steady state pulmonary diffusing capacity for carbon monoxide, 61.9 ± 14.8 per cent predicted ($P < 0.001$); arterial oxygen tension = 72.1 ± 13.6 mm Hg ($P < 0.001$); and hydrogen ion concentration = 36 ± 2.6 mμM per liter ($P < 0.001$). Carboxyhemoglobin and hemoglobin concentrations were similar in both groups.

A decrease in oxyhemoglobin affinity was observed (P_{50}: 28.0 ± 1.6 mm Hg versus control P_{50}: 25.8 ± 0.9 mm Hg; $P < 0.001$) without parallel increases in erythrocytic 2,3-DPG. Low, but significant, correlations between P_{50} and arterial oxygen tension, oxygen saturation, and hydrogen ion concentration, only, existed.

In contrast to certain patients with chronic obstructive airway disease, patients exhibiting a diffusion defect showed a rightward shift of the oxyhemoglobin dissociation curve without secondary polycythemia or significantly increased concentrations of 2,3-DPG. Mild hypoxemia might preclude increases in 2,3-DPG; however, P_{50} appeared to be a more sensitive index of oxyhemoglobin affinity. Both hydrogen ion concentration and reduced hemoglobin appeared to contribute to the regulation of P_{50}.

Introduction

In addition to secondary erythrocytosis and other cardiovascular and pulmonary defense mechanisms, qualitative adaptive changes that favor the unloading of oxygen at physiologic tensions develop rapidly within the erythrocyte in defense of tissue hypoxia. Thus, in certain clinical states associated with hypoxemia, the whole-blood oxygen dissociation curve shifts to the right of normal, rapidly and independently of the Bohr effect, providing a greater oxygen unloading for an identical change in arteriovenous oxygen tension difference. A presumptive causal relationship between this shift in oxygen-hemoglobin equilibria and increasing concentrations of intraerythrocytic organophosphates, particularly the glycolytic intermediates, 2,3-diphosphoglycerate (2,3-

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DPG) and to a lesser extent adenosine triphosphate (ATP) has been emphasized by several studies (1–3). Investigations focusing on hypoxemia secondary to chronic obstructive pulmonary disease have revealed a decreased hemoglobin affinity for oxygen as expressed by either the arterial oxygen tension at 50 per cent oxyhemoglobin saturation, 37°C, and pH 7.40 (P_{50}) or by independent measurements of 2,3-DPG (4–6). Simultaneous measurements of oxyhemoglobin affinity and erythrocytic organophosphate, however, are lacking except for the studies on ascent to altitude where a correlative increase in P_{50} and rapid increase in 2,3-DPG were observed (3). Additionally, the data of Lenfant and co-workers (7) reveal another interesting dissociation: patients with chronic obstructive lung disease showed rightward shift of the oxyhemoglobin dissociation curve only in conjunction with a hematocrit of 50 per cent or more.

The present study was designed to investigate P_{50} and simultaneous measurements of erythrocytic 2,3-DPG and ATP concentrations in persons with chronic arterial hypoxemia due to interstitial pulmonary fibrosis and granulomatosis in whom secondary erythrocytosis was absent because this relationship has not been studied previously in patients with pulmonary disease. The results indicate a decrease in affinity of hemoglobin for oxygen (increase in P_{50}) as a mechanisn for providing an adequate supply of oxygen to the tissues in patients with arterial hypoxemia due to sarcoidosis or interstitial fibrosis in the absence of secondary polycythemia. A parallel increase, however, in intraerythrocytic concentrations of 2,3-DPG or ATP was not observed.

Materials and Methods

Patient selection: Of the 21 subjects studied, pulmonary sarcoidosis was diagnosed in 18 (table 1). One patient was being treated with 10

TABLE 1

CLINICAL AND PHYSIOLOGIC DATA (MEAN ± SD) IN CONTROL SUBJECTS AND PATIENTS WITH PULMONARY SARCOIDOSIS

	Control Subjects	Pulmonary Sarcoidosis and Fibrosis	P
No.	12	21	
Age, years	28.8	34.9	
% White	41	20	
% Female	50	76	
% Smokers	33	38	
Mean duration of disease, months	–	54.1 ± 65	
FVC, % predicted	≥80	67.8 ± 17.6	
FEV_1/FVC, %	≥80	81.4 ± 7.4	
Hemoglobin, g/100 ml	13.5 ± 1.0	13.3 ± 2.7	
DL_{CO}, % predicted	≥80	61.9 ± 14.8	
Pa_{O_2}, mm Hg	90.0 ± 3.3	72.1 ± 13.6	
Sa_{O_2}, %	97.0 ± 1.0	93.2 ± 4.1	
Pa_{CO_2}, mm Hg	40.0 ± 0.8	36.8 ± 4.6	
$[H^+]$ nM/liter	39.8 ± 0.7	36.6 ± 2.6	
$P_{50_{7.40}}$, mm Hg	25.8 ± 0.9	28.0 ± 1.6	<0.001
$P_{50_{7.40}}$ corrected for CO, mm Hg	26.3 ± 0.9	28.6 ± 1.3	<0.001
2,3-DPG, $\mu M/10^{10}$ RBC	3.46 ± 0.89	3.73 ± 1.22	<0.5
ATP, $\mu M/10^{10}$ RBC	0.90 ± 0.30	0.75 ± 0.27	<0.9
HbCO, %	2.2 ± 1.9	2.7 ± 2.25	<0.5
pH, equivalent H^+	7.40	7.435	
Hill's *n*	2.5 ± 0.2	2.6 ± 0.2	<0.05

Definitions of symbols and abbreviations: $P_{50_{7.40}}$ = blood oxygen tension at 50 per cent oxyhemoglobin saturation, 37° C, and pH 7.40; 2,3-DPG = 2,3-diphosphoglycerate; ATP = adenosine triphosphate; HbCO = carboxyhemoglobin. Other symbols are standard.

mg prednisone every other day because of extensive parenchymal involvement for an eight-month period; all others were clinically stable. Three patients had clinically stable diffuse interstitial fibrosis although one patient with idiopathic fibrosis and one with chronic beryllosis were receiving maintenance corticosteroids (4 mg every other day and 8 mg every other day, respectively). All diagnoses were documented by lymph node biopsy, lung biopsy, or Kveim test. The mean age for the group was 34.9 years (range: 19 years to 62 years); 5 subjects were men and 16 were women, 38 per cent were smokers, and 80 per cent were nonwhite. The average duration from presentation to time of study was 54.1 ± 65 months with a range of 2 months to 252 months. Other known contributory factors to oxyhemoglobin affinity were excluded, i.e., sickle cell disease or other hemoglobinopathies, cirrhosis, congestive failure, or thyrotoxicosis. Twelve control subjects included 8 healthy nonsmokers (4 men) and 4 healthy smokers (2 men); 5 subjects were nonwhite. All P_{50} studies were performed before diffusion or spirometric testing and, where possible, cigarette smoking was discontinued six hours before the study.

Methods: Oxygen-hemoglobin affinity was quantified by the P_{50}, the partial pressure of oxygen (O_2) in mm Hg corresponding to 50 per cent oxyhemoglobin saturation at pH 7.40 and 37°C. An increased P_{50} reflects a decrease in the affinity of hemoglobin for O_2, or a rightward shift of the oxyhemoglobin dissociation curve. Fresh, heparinized arterial blood drawn anaerobically at rest was immediately assayed for oxygen tension (Pa_{O_2}), carbon dioxide tension (Pa_{CO_2}), and pH. Simultaneously, aliquots were equilibrated for 15 minutes in three vented, siliconized tonometers agitated at 37°C with humidified gas mixtures of 1.5 per cent, 3.0 per cent, and 5.0 per cent O_2, each containing 5.5 per cent carbon dioxide (CO_2), in nitrogen, at 6 liter per min flow. This yielded approximate tensions of 11.0 mm Hg, 21 mm Hg, and 36 mm Hg, respectively for O_2 and 40 mm Hg for CO_2.

A 3 wavelength spectrophotometric system (IL-182-CO-Oximeter),[4] was used for the analysis of total hemoglobin, oxyhemoglobin (HbO_2), and carboxyhemoglobin (HbCO). The spectrophotometer matrix was calibrated from blood samples of known hemoglobin saturation. The hemoglobin concentration and carbon monoxide (CO) saturation of the calibrating standard were measured and a well-mixed blood sample was then divided into three aliquots. The first was treated with sodium hydrosulfite for complete deoxygenated hemoglobin; the second was tonometered with a mixture of 95 per cent O_2 and 5 per cent CO_2 for full oxyhemoglobin saturation (the arterial oxyhemoglobin saturation—Sa_{O_2}—was extrapolated from the O_2 capacity as 100 per cent minus per cent CO saturation of the sample); and the third was equilibrated with 100 per cent CO. The absorbance of each species was converted to an electric signal, and the final results were read by equating voltages to each absorbance, as total hemoglobin concentration in g per 100 ml and per cent of total as oxyhemoglobin and per cent of total as carboxyhemoglobin. The standard deviation of the difference between results of Van Slyke-Neil manometric analysis and CO-oximeter was reported to be 0.81 per cent for O_2 saturation and 0.42 per cent for CO saturation. The standard deviation of O_2 saturation is 0.42 per cent and for CO is 0.41 per cent saturation by CO-oximeter measurements (8).

Frequent daily checks of the zero point were conducted by addition of freshly prepared sodium dithionite to 2 ml of arterial blood, and checks of the 100 per cent point, by 100 per cent O_2 in equilibrated blood, accounting for percentage HbCO.

After 15 minutes of equilibration in the tonometer, including syringe mixing every five minutes, assays were conducted for O_2 saturation and carboxyhemoglobin concentration in the CO-oximeter and for Po_2 Pco_2, and pH in the IL blood gas analyses Model No. 313[4] at 37°C. Duplicate or triplicate readings generally agreeing within 0.5 per cent for O_2 saturation and CO concentration, within 0.5 mm to 1.0 mm for Po_2 and Pco_2, and within 0.005 to 0.01 for pH were performed for each sample, and the results were averaged. The Po_2 was corrected for the Bohr effect (plasma pH variation) to a pH of 7.40 using the Severinghaus nomogram. The P_{50} was then determined graphically from a log-log plot of the three corrected Po_2-oxyhemoglobin saturation points. The best line, visually estimated through the three points, served as the basis for the graphic solution of P_{50}. When the points diverged widely, the entire study was repeated. The P_{50} at pH 7.40 was finally corrected for the Haldane-Smith effect due to the presence of carboxyhemoglobin in all smoking patients, using the formulation of Roughton and Darling: Po_2 corrected = Po_2 original $\left(1+\frac{Hb_{CO}}{Hb_{O_2}}\right)$, where Hb_{CO} is the carboxyhemoglobin satura-

[4] Instrumentation Laboratories, Waltham, Massachusetts.

tion and Hb_{O_2} is the oxyhemoglobin saturation. The corrected Po_2 corresponded to a saturation value equal to the sum of the hemoglobin saturation with O_2 and CO (9). Hill constant n was obtained from the slope of the plot log $Y/(1-Y)$ against log Pa_{O_2}, where Y equals the fraction of oxyhemoglobin. A decrease in n indicates a tendency for less heme-heme interaction.

Electrode calibrations for O_2 and CO_2 were made directly against a humidified gas mixture in which the concentrations were within the expected range of the study and which were measured by the Scholander microanalytic technique. All calibrated samples for Po_2 and Pco_2 agreed within ± 1.0 mm Hg, and within 0.005 units for pH, and all standard calibrations were performed immediately before each sample was assayed.

Hemoglobin was determined spectrophotometrically; erythrocyte counts were performed in a Coulter counter. Simple pulmonary mechanics were obtained with a standard spirometer; values were expressed as BTPS. Pulmonary diffusing capacity for carbon monoxide (D_{LCO}) was determined by the steady state technique (Filley) using 0.1 per cent CO in room air and was corrected for CO capillary pressure; normal values were obtained from Bates and Christie (10). The diffusion tests were generally performed within one year of the present study and did not coincide with P_{50} or determination of mechanics of breathing. All other diffusion and spirometry studies were performed after P_{50} determinations.

A modification of the method of Krimsky (11) was used to determine 2,3-DPG. A neutralized trichloroacetic acid extract was diluted in distilled water, and 0.06 ml was added to a 3.01-ml of 0.04 *M* Tris buffer, pH 7.4, containing 0.01 *M* magnesium chloride; 0.025 *M* phosphoenolpyruvate[5]; 0.05 mg enolase (muscle preparation)[5], 0.05 mg phosphoglycerate mutase (muscle preparation).[5] Change in absorbance was measured in a Beckman DU spectrophotometer. A standard curve was made, and results were expressed in μM per 10^{10} erythrocytes.

Adenosine triphosphate was measured with the reagents of Sigma Kit No. 366[6] according to directions in the technical bulletin. Results were calculated using the extinction coefficient for reduced nicotinamide adenine dinucleotide and were expressed as μM per 10^{10} erythrocytes.

TABLE 2

CORRELATION (r) BETWEEN P_{50}, 2,3-DPG, AND OTHER VARIABLES IN PATIENTS WITH PULMONARY SARCOIDOSIS

	n*	r
$P_{50_{7.40}}$ versus		
Pa_{O_2}	21	−0.44
Sa_{O_2}	21	−0.47
$[H^+]$	21	−0.44
$P_{50_{7.40}}$ corrected	20	+0.89
2,3-DPG	21	+0.12
Hemoglobin concentration	21	−0.13
ATP	20	−0.48
D_{LCO}	18	−0.11
Duration of disease	21	+0.05
FVC	21	−0.36
Bicarbonate concentration	21	+0.22
DPG versus		
Pa_{O_2}	21	+0.31
Sa_{O_2}	21	+0.28
$[H^+]$	21	+0.31
Hemoglobin concentration	21	+0.02
D_{LCO}	18	−0.08
Duration of disease	21	−0.33
HbCO	20	+0.45

*n = number of observations.

See table 1 for definitions of symbols and abbreviations.

Results

Physiologic parameters: The values for spirometry, diffusion, and arterial blood gases were consistent with a restrictive process (reduced lung compliance), and impaired diffusion capacity with arterial hypoxemia, mild oxyhemoglobin desaturation, and hyperventilation with a partially compensated respiratory alkalosis. The difference in mean hemoglobin and HbCO concentrations between control subjects and patients was not significant ($P < 0.8$ and $P < 0.5$, respectively) (table 1).

P_{50} and Hill constant (n): The mean (± SD) P_{50} of 28.0 ± 1.6 mm Hg in the group of 21 patients was significantly greater than that in the control subjects, 25.8 ± 0.9 ($P < 0.001$). The correlation coefficient (r) between P_{50} and Pa_{O_2} of −0.44 was significant at the $P < 0.02$ level. Similarly, a low but significant correlation was observed between P_{50} and Sa_{O_2} ($r = -0.47$; $P < 0.02$) and P_{50} with hydrogen ion concentration (H^+) ($r = -0.44$; $P < 0.02$). No correlation was observed between P_{50} and

[5] Calbiochem, San Diego, California.

[6] Sigma Chemical Company, St. Louis, Missouri.

concentration of 2,3-DPG ($P < 0.5$), hemoglobin concentration ($P < 0.5$), per cent of predicted D_{LCO} ($P < 0.6$), duration of disease ($P < 0.8$), or the per cent of predicted forced expiratory volume ($P < 0.1$). There was a negative correlation between P_{50} and erythrocytic ATP of $r = -0.48$ ($P < 0.02$) (tables 1 and 2).

Hill constant, n, was 2.5 with a standard deviation of 0.2 in the normal subjects whereas a significantly greater value of n, 2.6 ± 0.2, was present in the patient population ($P < 0.05$) (table 1).

Any effect of corticosteroids on P_{50} could not be identified in the present data despite reported, steroid related, rightward shifts (12).

Erythrocytic organic phosphate changes: The mean (± SD) concentrations of 2,3-DPG and ATP of 3.73 ± 1.22 and 0.75 ± 0.27 μM per 10^{10} erythrocytes, respectively, were not statistically different from the normal control values ($P < 0.5$ and $P < 0.9$) (tables 1 and 2). Among the sarcoid patients, no correlations were observed between 2,3-DPG and Pa_{O_2}, Sa_{O_2}, H^+, HCO_3, D_{LCO}, P_{50}, hemoglobin concentration, or duration of disease. A partial relationship between 2,3-DPG and HbCO was suggested by the correlation coefficient of +0.45 ($P < 0.02$).

Effects of smoking and carbon monoxide levels: The mean carboxyhemoglobin concentrations of the control group and the patients of 2.2 ± 1.9 per cent and 2.7 ± 2.25 per cent, respectively, were not significantly different ($P < 0.5$) (table 1). Within the patient group, there was a significant increase in HbCO among smokers ($P < 0.01$), averaging 4.2 ± 2.7 per cent (table 3). When P_{50} was further corrected for HbCO effect, a significant increase was observed ($P < 0.001$); 26.3 ± 0.9 and 28.6 ± 1.3 mm Hg in the patient group and control groups, respectively. Once corrected, the P_{50} was equivalent in nonsmokers and smokers. There were no differences in 2,3-DPG, ATP, or hemoglobin concentrations between smokers and nonsmokers among the patient population. Similarly, when both nonsmokers and smoking patients were subdivided, no correlations between P_{50} (corrected or uncorrected) and hemoglobin concentration or 2,3-DPG were observed (table 3).

Discussion

In patients with pulmonary disorders, it is important to identify the adaptive and regulatory mechanisms that ensure an adequate supply of cellular oxygen under conditions of arterial hypoxemia. Thus a rightward shift of the oxyhemoglobin dissociation curve, which favors the unloading of oxygen from erythrocytes to tissue, might be an important compensatory mechanism in patients with systemic hypoxemia due to chronic obstructive lung disease (4–6). Similar responses exist on ascent to altitude, in congenital, cyanotic heart disease, in congestive heart failure, and with various anemias (4, 13–15). The intraerythrocytic organic phosphate, 2,3-DPG, which accounts for 75 per cent of total organic erythrocytic phosphates, and to a lesser extent ATP, appear to exert an important causal effect in displacing the position of the O_2-Hb dissociation curve. 2,3-DPG is synthesized from 1,3-DPG during glycolysis in the erythrocyte by the activity of 2,3-diphosphoglycerate mutase; this enzyme is easily inhibited by its reaction product, 2,3-DPG. The latter organophosphate combines specifically and reversibly with the hemoglobin tetramer in the deoxyhemoglobin configuration and reduces the product inhibition permitting greater synthesis of 2,3-DPG. Under conditions of hypoxia and increased deoxyhemoglobin concentrations, more binding of 2,3-DPG occurs, thereby perhaps reducing the inhibition of the 2,3-diphosphoglycerate mutase with resultant augmented synthesis of 2,3-DPG. This further decreases the affinity of hemoglobin for oxygen (2, 3, 16, 17). The effect of 2,3-DPG on the dissociation curve is distinct from the effect of hydrogen ion (Bohr). Because alkalemia stimulates intraerythrocytic 2,3-DPG synthesis, and acidemia is inhibitory, the direct effect of pH on 2,3-DPG is opposite that of the Bohr effect and for a given set of clinical conditions an interplay of acid-base status and erythrocytic organophosphate effect will influence the final position of the oxyhemoglobin curve and thus delivery of O_2 (18).

TABLE 3

DIFFERENCES IN MEAN (± SD) VALUES BETWEEN SMOKERS AND NONSMOKERS (PATIENT GROUP)

	Smokers	Nonsmokers	P
2,3-DPG, $\mu M/10^{10}$ RBC	4.2 ± 1.4	3.4 ± 1.1	NS*
Hemoglobin, g/100 ml	14.0 ± 3.2	13.8 ± 2.25	NS
$P_{50_{7.40}}$, mm Hg	26.9 ± 0.9	28.7 ± 1.6	<0.01
HbCO, %	4.2 ± 2.7	1.5 ± 0.8	<0.01
$P_{50_{7.40}}$, corrected, mm Hg	28.1 ± 0.9	28.9 ± 1.4	NS

*NS = not significant.

See table 1 for definitions of symbols and abbreviations.

The present study examined both P_{50} and erythrocytic organophosphate changes in a relatively homogenous clinical-physiologic population, those with diffuse interstitial granulomatosis or fibrosis. These patients with a reduced vital capacity and lung compliance, and a diffusion defect generally exhibited modest hypoxemia and minor degrees of hypocapnia without secondary polycythemia. An increase in P_{50} was observed, in agreement with the observations at altitude and of certain patients manifesting chronic obstructive pulmonary disease. The subjects, however, contrasted with those with obstructive pulmonary disease studied by Lenfant and associates (7) in whom more severe hypoxemia (mean Pa_{O_2}: 47.5 ± 8.7 mm Hg) and chronic, stable, hypercapnia (mean Pa_{CO_2}: 51.1 ± 7.3 mm Hg) was present and who appeared to fall into two groups: one exhibited an increase in P_{50} as well as polycythemic response (hematocrit greater than 50 per cent) and the other no change or a leftward shift when the hematocrit was 50 per cent or less; Pa_{O_2} and Pa_{CO_2} were approximately equal in both groups (7). Inasmuch as patients with pulmonary granulomatosis and fibrosis demonstrate a rightward shift with a normal hematocrit, other parameters such as electrolyte and ionic strength changes, intracellular pH factors or severity of hypoxemia might play a role in chronic obstructive airway diseases in influencing affinity of hemoglobin for oxygen (19). Such P_{50} differences in relation to polycythemia have not been entirely clear from other studies of obstructive lung disease. Evidence for decreased affinity for O_2, however, is substantiated; 5 patients with unspecified chronic lung disease (mean Sa_{O_2}: 84.6 per cent) exhibited significantly increased concentrations of 2,3-DPG, but hematocrit was not specified (4), and all 5 patients with secondary polycythemia (hematocrit greater than 50 per cent) due to chronic obstructive lung disease had a mean P_{50} of 29.8 mm Hg (6).

Measurements of erythrocytic 2,3-DPG and ATP in sarcoidosis and pulmonary fibrosis failed to reveal a trend similar to the increased P_{50} response; mean organophosphate concentrations were no greater than those of the control group. Subdivision of the patients into groups of greater and lesser degrees of hypoxemia revealed no differences. The chronicity of disease would eliminate, as a factor, the time required for 2,3-DPG to increase. There were, however, 5 patients who exhibited concentrations of 2,3-DPG greater than 4.5 μM per 10^{10} erythrocytes (mean: 5.2 μM per 10^{10} erythrocytes), but corresponding P_{50} values in this group were scattered and neither anemia nor H^+ ion changes appeared distinctive. In the patient group, there was a negative, but low, correlation between P_{50} and hydrogen ion activity (table 2); however, the known relationship between decreasing H^+ activity and increased concentrations of 2,3-DPG was not observed despite the rightward displacement of the oxyhemoglobin curve (P_{50}). These observations are in contrast to those of ascent to altitude (15,000 feet) where hypoxemia and respiratory alkalemia develop and a significant and rapid increase in 2,3-DPG is paralleled by an increase in P_{50} (3). It is probable, however, that lesser degrees of hypocapnia and respiratory alkalemia were present in our sarcoid group. In patients with congenital heart disease (more

than three months of age), a relationship between Pa_{O_2} and 2,3-DPG was evident only when the Pa_{O_2} was less than 60 mm Hg; tensions greater than 60 mm Hg were associated with normal erythrocytic 2,3-DPG (20). In addition, rats exposed to mild hypoxia produced by breathing 18 per cent O_2 over a 24-hour period exhibited essentially no effect on 2,3-DPG concentrations; however, lower inspired concentrations were followed by a steep and linear increase in 2,3-DPG (21). The present patients on the whole exhibited only mild degrees of hypoxemia and oxyhemoglobin desaturation ($Sao_2 = 93.2 \pm 4.1$ per cent), albeit occasionally more severe than in the rat study cited, and a small, but significant, shift in P_{50}. Thus, mild degrees of arterial hypoxemia due to a diffusion barrier or ventilation/perfusion imbalance might not have induced sufficient change in 2,3-DPG concentrations to permit differences or correlations between these two parameters to become discernible. The evident shift in P_{50}, however, suggests that it might be a more sensitive index of hypoxia and might in part be consistent with the recent observations of Shappell and co-workers (22), which showed a reduced affinity of hemoglobin for oxygen within minutes in coronary sinus blood during atrial pacing in patients with angina pectoris without measurable changes in the factors known to influence P_{50}.

The exact cause of such shifts in oxygen-hemoglobin equilibria is currently under investigation. Reduced oxyhemoglobin affinities in anemia occur despite normal arterial oxygen tensions and saturations. Several studies relate oxyhemoglobin curve displacements to the low oxygen saturation of the mixed venous blood ($S\bar{v}_{O_2}$); here the duration and ratio of reduced hemoglobin to oxyhemoglobin (time average $Hb\text{-}Hb_{O_2}$ ratio) are implicated as one major regulatory factor (7). This concept has been supported in part by observations of P_{50}, 2,3-DPG, and $S\bar{v}_{O_2}$ in cardiac failure (13). Patients with pulmonary induced hypoxemia, anemia, or heart failure would theoretically exhibit a low $S\bar{v}_{O_2}$ as a common denominator. That other factors may be implicated is supported by the observation of an increase in P_{50} without changes in 2,3-DPG during atrial pacing (22). Additionally, Woodson and associates (13) have pointed out that in heart failure the correlation of Sv_{O_2} with 2,3-DPG and P_{50}, while statistically significant, is not absolute. The importance of erythrocytic pH (time average pH) as another critical factor in the regulation of oxygen-hemoglobin affinity was recently stressed by Astrup (18). Although the exact basis for control of oxygen-hemoglobin affinity and concentrations of 2,3-DPG is yet to be clarified, both reduced hemoglobin concentrations and erythrocytic pH appear to be important determinants. The low correlations between P_{50} and arterial oxygen tension (r: −0.44), oxyhemoglobin saturation (r: −0.47), and pH (r: −0.44) in sarcoidosis and pulmonary fibrosis are in accord with some of the known factors influencing hemoglobin-oxygen affinity. Inasmuch as arterial hypoxemia could reflect increasing concentrations of reduced hemoglobin, the present observations are consistent with the concept of mixed venous blood oxygen saturation as a factor in this regulatory mechanism.

Although the pathophysiologic mechanism of hypoxemia is presumably different in obstructive airway disease and restrictive disorders with decreased D_{LCO}, the net effect, arterial hypoxemia, is the same and the observation of a leftward shift in persons with obstructive lung disease and normal hematocrit is contrary to that seen in sarcoidosis. These differences might reflect compensatory cardiopulmonary and hemoglobin responses for appropriate oxygen delivery to the tissues in a given clinical state, somewhat analogous to the situation with hemoglobin Seattle where a rightward oxyhemoglobin displacement might provide an adequate tissue oxygen supply despite the anemia (23). It would currently appear that patients with chronic obstructive airway disease or pulmonary fibrotic-granulomatous parenchymal disease of diverse origins respond to hypoxemia in part by (*1*) a rightward shift of the oxyhemoglobin dissociation curve (increase in P_{50}) with or without an increase in erythrocytic 2,3-DPG, (*2*) secondary erythrocytosis, or (*3*) a combination of these mechanisms. Persons failing to ex-

hibit such changes presumably do not experience tissue hypoxia or have failed to adapt for reasons unclear at present. The chronically hypoxemic patient with pulmonary sarcoidosis or fibrosis does not generally exhibit secondary polycythemia but does adapt by a rightward displacement of the oxygen dissociation curve in the absence of significant increments in erythrocytic organophosphate.

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RESUMEN

Afinidad oxihemoglobínica en la granulomatosis pulmonar crónica (sarcoidosis) y en la fibrosis

La afinidad oxihemoglobínica fué evaluada en 21 pacientes con hipoxemia crónica arterial debido a fibrosis pulmonar y a granulomatosis (sarcoide) por medidas de la tensión de oxígeno arterial bajo saturación oxihemoglobínica de 50 por ciento, 37°C, y pH 7.40 (P_{50}), eritrocítico 2,3-difosfoglicerato (2,3-DPG) y trifosfato adenosino eritrocítico. Doce personas normales sirvieron como sujetos de control. Los valores fisiológicos medios que caracterizaban a estos pacientes incluyeron: capacidad vital forzada = 67.8 ± 17.6 por ciento de lo pronosticado ($P < 0.01$); capacidad de difusión pulmonar de estado estable para el monóxido de carbón = 61.9 ± 14.8 por ciento de lo pronosticado ($P < 0.001$); concentración ion de hidrógeno = 36.6 ± 2.6 mμM por litro ($P < 0.001$). Las concentraciones de carboxihemoglobina y de hemoglobina fueron similares en ambos groupos.

Se observó una disminución en la afinidad de exihemoglobina (P = 28.0 ± 1.6 mm Hg contra el control P_{50} = 25.8 ± 0.9 mm Hg; $P < 0.001$) sin aumentos paralelos en eritrocíticos 2,3-DPG. Existieron correlaciones bajas, pero significantes entre el P_{50} y la tensión de oxígeno arterial, la saturación de oxígeno arterial y la concentración ion de hidrógeno, únicamente.

En contraste con algunos pacientes con enfermedad crónica obstructiva de los conductos respiratorios, los pacientes que mostraron un defecto de difusión mostraron un cambio hacia la derecha de la curva de disociación de oxihemoglobina sin policitemia secundaria o concentraciones de 2,3-DPG significantemente aumentadas. La hipoxemia leve puede precluir aumentos en el 2,3-DPG; sin embargo, el P_{50} parece ser un índice de afinidad oxihemoglobínica más sensitivo. La concentración ion de hidrógeno y la hemoglobina reducida parecieron contribuir a la regulación del P_{50}.

RESUME

Affinité de l'oxyhémoglobine dans la granulomatose pulmonaire chronique (sarcoidose) et dans la fibrose pulmonaire

Chez 21 malades souffrant d'hypoxémie artérielle chronique due à une fibrose pulmonaire et à une granulomatose (sarcoïde), on a procédé à une évaluation de l'affinité pour l'oxyhémoglobine, en mesurant la tension artérielle en oxygène à 50 pour cent de saturation en oxyhémoglobine à 37° C et pH 7,4 (P 50), la teneur en 2,3 diphosphoglycérate érythrocytaire (2,3-DPG), et l'adénosine triphosphate des érythrocytes. Douze individus normaux ont servi de sujets témoins. Les valeurs physiologiques moyennes qui caractérisaient ces malades étaient les suivantes: capacité vitale forcée = 67,8 ± 17,6 pour cent des valeurs de prédiction ($P < 0,01$), capacité de diffusion pulmonaire à l'état stable pour le monoxyde de carbon = 61,9 ± 14,8 pour cent des valeurs de prédiction ($P < 0.001$); tension artérielle en oxygène = 72,1 ± 13,6 mm Hg ($P < 0.001$); concentration en ions hydrogène = 36,6 ± 2,6 mμM par litre ($P < 0.001$). Les concentrations en carboxyhémoglobine et en hémoglobine étaient similaires dans les deux groupes.

Une diminution de l'affinité pour l'oxyhémoglobine a été observée (P 50 = 28,0 ± 1,6 mm Hg, par rapport aux valeurs observées P 50 chez les témoins = 25,8 ± 0,9 mm Hg; ($P < 0.001$), et ceci sans qu'une élévation parallèle soit notée dans la 2,3-DPG érythrocytaire. Des corrélations faibles, mais néanmoins significatives, ont été relevées entre la P 50 et la tension artérielle en oxygène, la saturation artérielle en oxygène, et la concentration en ions hydrogènes; aucune corrélation n'a été notée en ce qui concerne les autres paramètres.

Contrairement à ce qu'on observe chez certains malades souffrant d'une affection obstructive chronique des conduits aériens, les sujets présentant des troubles de la diffusion ont montré un glissement vers la droite de la courbe de dissociation de l'oxyhémoglobine, sans polycythémie secondaire et sans élévation significa-

tive dans les concentrations de 2,3-DPG. Une hypoxémie légère peut empêcher une augmentation de la 2,3-DPG. Néanmoins, la P 50 semble constituer un index plus sensible de l'affinité pour l'oxyhémoglobine. La concentration en ions hydrogènes et une réduction en hémoglobine semblent contribuer à la fois à la régulation de la P 50.

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