Oxyhemoglobin Affinity in Bronchial Asthma: Chronic Stable State, Acute, and Status Asthmaticus*

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Studies in chronic stable bronchial asthma revealed normal $P_{50_{7.40}}$; COLD controls exhibited significantly elevated $P_{50_{7.40}}$ and 2,3-DPG levels. In patients with acute asthma, no differences in $P_{50_{7.40}}$ or 2,3-DPG were discernible. Subdivision of acute asthma into categories with hyperventilation ($PaCO_2 \leq$ 37.0 mm Hg) and hypoventilation ($PaCO_2 \geq$ 37.1 mm Hg) revealed that hyperventilating patients exhibited a mean $P_{50_{7.40}}$ of 27.7 $\pm$ 4.3 mm Hg unchanged over normal controls or chronic stable asthma while hypoventilating subjects were significantly left-shifted ($P_{50_{7.40}} = 25.1 \pm 0.7$ mm Hg) contrasted with normal and stable asthmatic patients ($P < 0.001$) despite significant oxyhemoglobin desaturation (80.2 percent). 2,3-DPG data paralleled these observations. The pH was found to influence the P_{50} change in the hypoventilating population. MCHC measurements, percent HbCO or hemoglobin was not related. While the Bohr effect, reflected in estimates of physiologic in vivo P_{50} values, tends to temper leftward shifts in asthma associated with respiratory acidosis, the lack of compensatory increase in 2,3-DPG and an associated elevated $P_{50_{7.40}}$, in spite of the hypoxic stimulus, may limit maximal oxygen delivery to tissues.

Under clinical conditions associated with hypoxia a rightward shift of the whole-blood oxyhemoglobin dissociation curve develops and is postulated to reflect an adaptive mechanism resulting in an increased unloading of oxygen. The intraerythrocytic organophosphate 2,3-diphosphoglycerate (2,3-DPG) appears to play a causal role in this relationship.[1-3] Previous reports in a variety of pulmonary disorders, such as stable state chronic bronchitis, pulmonary emphysema, and chronic, stable pulmonary granulomatosis and fibrosis have supported this shift in oxyhemoglobin affinity.[1,2,4-6] The present study was designed to investigate oxygen-hemoglobin affinity in conjunction with 2,3-DPG measurements during chronic stable bronchial asthma and to contrast further these observations during *acute* episodes, including status asthmaticus.

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Materials and Methods

Patient Selection

Twenty-eight patients with bronchial asthma were selected on the basis of a clinical history of continuous or episodic wheezing and dyspnea, allergic diathesis, airways obstructive disease pattern with at least a 15 percent response in FVC or $FEV_{1.0}$ percent following aerosol isoproterenol therapy, dermal reactivity to standard allergens, blood and sputum eosinophilia, and the absence of chronic bronchitis or pulmonary emphysema by clinical, radiologic and physiologic features. Patients with acute or chronic, stable asthma were defined by the clinical history, spirometry and blood gas changes; the acute or status asthmaticus state was also qualitatively graded as to severity by a 1 to 4+ (4+ = status asthmaticus) score by these same factors. In some cases more than one acute study was conducted in a given patient but this was always a different episode (Table 1). No patient had a history of congestive heart failure, anemia, thyrotoxicosis, alcoholism or known hemoglobinopathy and no attempt was made to control cigarette consumption. Medications were not altered during the study, and as a group the following were being employed: aminophylline preparations, aerosol isoproterenol, glyceryl guiacolate, adrenal corticosteroids, a combination of theophylline, ephedrine and phenobarbital (Tedral), various antihistamine preparations and antimicrobials.

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Table 1—*Pertinent Clinical and Laboratory Data: Control and Asthmatic Populations.

	Normal Controls	Bronchial Asthma		Chronic Obstructive Lung Disease Controls
		Stable	Acute	
No.	17	26	12	12
Age (yr.)	28.5 ± 7.7	43.8 ± 17.4	39.0 ± 15.9	64.3 ± 9.1
Women, %	48	71	65	9
Smokers, %	41	9	16	45
Mean duration hr.	—	—	43.2 ± 24.3	—
days	—	12.6 ± 2.8	—	—
FVC (% predicted)	>80%	67.1 ± 17.1	49.5 ± 20.4	—
FEV 1.0 (% observed)	>75%	64.3 ± 12.4	52.3 ± 13.3	—
Hgb (gm %)	13.8 ± 1.2	13.4 ± 1.7	14.2 ± 3.0	15.2 ± 2.4
PaO_2 (mm Hg)	90.0 ± 3.9	79.1 ± 10.4	55.6 ± 2.8	61.5 ± 11.1
SaO_2 (%)	96 0 ± 1.0	95.1 ± 1.6	84.6 ± 7.9	89.0 ± 6.9
$PaCO_2$ (mm Hg)	39.8 ± 1.3	35.6 ± 3.6	38.1 ± 8.4	48 9 ± 10.9
pHa	7.40 ± 0 01	7.44 ± 0.03	7.39 ± 0.07	7.41 ± 0.04
(H^+) (nM/L)	39.8 ± 0.9	36.3 ± 2.4	40.7 ± 7.0	38.9 ± 3.4
HCO_3^- (mEq/L)	23.0 ± 2.0	24 0 ± 2.5	22.1 ± 3.3	29.8 ± 6.1
$P_{50_{7.40}}$ (mm Hg)	26.2 ± 1.0	27.2 ± 1.6	26.0 ± 2.7	28.8 ± 2.2
$P_{50_{7.40}}$ (corrected CO)	26.8 ± 0.3	27.6 ± 1.6	26.4 ± 3.1	29.9 ± 2.3
2,3-DPG ($\mu M/10^{10}$ RBC)	3.44 ± 0.79	3.82 ± 1.32	3.17 ± 1.28	4.19 ± 1.48
ATP ($\mu M/10^{10}$ RBC)	0.97 ± 0.35	0.94 ± 0.31	0.99 ± 0.65	0.79 ± 0.35
HbCO, %	2.5 ± 2.1	1.6 ± 0.9	1.7 ± 1.82	3.3 ± 3.1
Mean severity, 1-4+	—	≤1.0	3.0	—
Hill's "n"	2.62 ± 0.31	2.69 ± 0.23	2.81 ± 0.12	2 71 ± 0.47
MCHC	31.75 ± 1.61	31.67 ± 2.15	31.40 ± 1.93	32 76 ± 2.85

*Mean ± 1 SD

Two groups of subjects served as controls (Table 1). In the first instance 17 normal volunteers without any complicating disorders known to influence oxyhemoglobin affinity were selected. A second type of control consisted of 12 patients with chronic obstructive lung disease (COLD) and significant arterial hypoxemia who were studied during the same period as the asthmatic population.

All blood sampling was performed prior to spirometric studies or oxygen administration. Where possible, the stable to acute phase asthmatic observations were performed in a paired fashion; that is the chronic stable patient serving as the control for the acute asthmatic attack study.

Methods

Oxygen-hemoglobin affinity was quantitated by the P_{50}, the partial pressure of O_2 in mm Hg corresponding to 50 percent oxyhemoglobin saturation at pH 7.40 and 37°C. An increased P_{50} reflects a decrease in the affinity of hemoglobin for oxygen, or a rightward shift of the oxyhemoglobin dissociation curve. Fresh, heparinized arterial blood drawn anaerobically at rest (after three to five minutes to minimize hyperventilation) was immediately assayed for PaO_2, $PaCO_2$, 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 percent, 3.0 percent, and 5.0 percent oxygen, each containing 5.6 percent carbon dioxide and the balance nitrogen, at 6 L/min flow. This yielded approximate *in vitro* tensions of 11, 21 and 36 mm Hg respectively for oxygen and 40 mm Hg for CO_2.

A three wavelength spectrophotometric system (IL-182-CO-oximeter, Instrumentation Laboratory) was employed for analysis of total hemoglobin, oxyhemoglobin (HbO_2) and carboxyhemoglobin (HbCO). The spectrophotometer matrix is calibrated from blood samples of known hemoglobin saturation by methods previously described.[6,7] Frequent daily checks of the zero point were conducted by addition of freshly prepared sodium dithionite to 2 ml of arterial blood, and of the 100 percent point by 100 percent oxygen in equilibrated blood, accounting for percentage HbCO.

Following 15 minutes' equilibration in the tonometer, including syringe mixing each five minutes, assays were conducted for oxygen saturation and carboxyhemoglobin concentration in the CO-oximeter and for P_{O_2}, P_{CO_2} and pH in the Instrumentation Laboratories blood gas analyses model no. 313 at 37°C. Duplicate or triplicate readings generally agreeing within 0.5 percent for oxygen saturation and carbon monoxide concentration, and 0.5 to 1.0 mm for P_{O_2} and P_{CO_2} and within 0.005 to 0.01 for pH were performed for each sample and the results averaged. The P_{O_2} was corrected for the Bohr effect (plasma pH variation) to a pH of 7.40 by employing the Severinghaus nomogram.[8] The $P_{50\,7.40}$ was then determined graphically from a log-log cycle plot of the three corrected P_{O_2}-oxyhemoglobin saturation points employing the y axis for $\frac{\text{Percent } O_2 \text{ saturation}}{\text{100-percent saturation}}$ and corrected P_{O_2} directly on the abscissa. The best line, visually estimated through the three points, served as the basis for the graphic solution of $P_{50\,7.40}$. In the presence of widely divergent points the entire study was repeated. The P_{50} at pH 7.40 value was finally corrected for the Haldane-Smith effect in all patients who smoked cigarettes, due to the presence of carboxyhemoglobin, employing the formulation of Roughton and Darling: P_{O_2} corrected = P_{O_2} original $\left(1 + \frac{HbCO}{HbO_2}\right)$ the corrected PO_2 corresponding to a saturation value equal to the sum of the hemoglobin saturation with oxygen and carbon monoxide.[9] Hill's constant "n" was obtained from the slope of the plot log $\frac{Y}{1-Y}$ against log PaO_2 where Y equals the fraction of oxyhemoglobin. A decrease in "n" indicates a tendency for less heme-heme interaction. Physiologic, *in vivo*

Table 2—*Comparison of Controls and Patient Population: Selected Parameters.*

Group: Parameter	Stable Asthma Vs Acute Asthma*	Stable Asthma Vs Normals	Acute Asthma* Vs Normals	Normals Vs COLD†
$P_{50_{7.40}}$ (corrected CO)	NS	NS	NS	S, p<0.001‡
PaO_2, mm Hg	S, p<0.001	S, p<0 001	S, p<0.001	S, p<0.001
SaO_2, %	S, p<0.001	S, p<0 001	S, p<0.001	S, p<0.001
$PaCO_2$, mm Hg	NS	S, p<0.001	NS	S, p<0.01
pH	S, p<0.001	S, p<0.001	NS	NS
HCO_3^-, mEq/L	NS	NS	NS	S, p<0.001
Hgb, gm %	NS	NS	NS	S, p<0.05
2,3-DPG, $\mu M/10^{10}$ RBC	NS	NS	NS	S, p=0.05
ATP, $\mu M/10^{10}$ RBC	NS	NS	NS	NS
HbCO, %	NS	NS	NS	NS
FVC, % predicted	S, p<0.05	—	—	—
FEV 1.0%	S, p<0.05	—	—	—
Age	NS	S, p<0.01	S, p<0.02	S, p<0.001
MCHC	NS	NS	NS	NS

NS = Non-significant difference
S = Significant difference, p given
† = COLD
* = All acute asthmatics combined
‡ = Not shown P_{50} (corrected) COLD vs acute and stable asthma: Significant difference p<0.001

P_{50}, accounting for the Bohr effect, was determined by converting the final *mean* $P_{50\,7.40}$ value by the relationship: $\Delta \log P_{50}/\Delta pH = -0.48$, where ΔpH is based on the *actual* plasma pH.[10]

Oxygen and carbon dioxide electrode calibrations were made directly against humidified commercial gas mixtures having concentrations of about 5.3 percent CO_2 and up to 12.0 percent O_2, balance nitrogen were within the expected range of the study and which agreed to 0.06 percent as measured by the Scholander microanalytic technique. All calibrated samples for P_{O_2} and P_{CO_2} agreed within H± 1.0 mm Hg, and 0.005 units for pH and all standard calibrations were performed immediately before each sample was assayed.

Hemoglobin was determined spectrophotometrically; red blood cell counts were performed in the Coulter Counter, model B, and are expressed as number of RBC × 10^6 per cu mm; microhematocrit was determined after five minutes and mean corpuscular hemoglobin concentration (MCHC) is expressed as gm Hb/100 ml red cells; simple pulmonary mechanics were obtained from a standard spirometer (BTPS).

A determination of 2,3-diphosphoglycerate was made by a modification of the method of Krimsky.[11] A neutralized trichloroacetic acid extract was diluted in distilled H_2O, and 0.06 ml was added to a 3.01 ml volume containing tris buffer (pH 7.4) 0.04 M, $MgCL_2$ 0.01M, phosphoenolpyruvate 0.025M, enolase (commercial muscle preparation) 0.05 mg, phosphoglycerate mutase (commercial muscle preparation) 0.05 mg.* Change in O.D. was measured on the Beckman DU. A standard curve was made and the results expressed in $\mu M/10^{10}$ red cells. Adenosine triphosphate (ATP) was measured with the reagents of Sigma kit no. 366 according to directions in the technical bulletin. Results were calculated using the extinction co-efficient for DPNH and are expressed as $\mu M/10^{10}$ red cells.

Results

Clinical-Physiologic Parameters

The mean (± 1 SD) values of pertinent clinical and physiologic data presented in Tables 1 and 2 indicate the nature of the asthmatic population. Chronic stable bronchial asthma was characterized by mild hypoxemia, hyperventilation and respiratory alkalosis. These patients were older than normal controls, but hemoglobin and HbCO levels were similar. In the acute attack (2.9 ± 0.9 + severity) greater hypoxemia and oxyhemoglobin desaturation (nomogram) developed. The mean pH of 7.39 ± 0.07 in the acute attack was significantly different from the pH of 7.44 ± 0.03 in the stable state (P<0.001). $PaCO_2$ *averaged* the same for acute asthma and normal subjects. Neither age, hemoglobin nor percent HbCO were different among stable state versus acute asthmatics. The duration of the acute attack was 43.2 ± 24.3 hours. Analysis of paired observations, that is, a stable state serving as the control for an acute attack in the same patient, revealed a significant fall in PaO_2 and SaO_2 (P<0.001), HCO_3- (P<0.05) and hemoglobin concentration (P<0.05); $PaCO_2$, pH and HbCO were not significantly different.

The COLD patients, included as controls during the study period, exhibited similar degrees of arterial hypoxemia (PaO_2 = 48.9 ± 10.9 mm.Hg).

$P_{50\,7.40}$ (corrected for CO) and Hill's Constant (n)

The mean $P_{50\,7.40}$ in 26 *stable* asthmatics of 27.6 ± 1.6 mm Hg was not significantly different from normal subjects, 26.8 ± 0.3 mm Hg, or acute asthmatics, 26.4 ± 3.1 mm Hg. Similarly, the mean $P_{50\,7.40}$ in *acute* asthma was not different from normal or stable asthma. Additionally, paired data analysis revealed no difference in $P_{50\,7.40}$ in the acute attack compared with the chronic stable state

*The latter three reagents were obtained from Calbiochem.

Table 3—*Changes in Acute Bronchial Asthma Compared with Stable State Observations in the Same Patient: Paired Analysis.*

Parameter	Observations, No.	Significance
$P_{50_{7.40}}$, corrected CO	12	NS
PaO_2, mm Hg	11	S, <0.001
SaO_2, %	11	S, <0.001
$PaCO_2$, mm Hg	12	NS
pH	11	NS
HCO_3^-, m Eq/L	12	S, <0.05
Hgb, gm %	14	S, <0.05
2,3-DPG, $\mu M/10^{10}$ RBC	13	NS
ATP, $\mu M/10^{10}$ RBC	12	NS
HbCO, %	13	NS

(Table 3). However, the mean $P_{50_{7.40}}$ for COLD patients of 29.9 ± 2.3 mm Hg *was* significantly greater than both normal and asthmatic subjects ($P<0.001$) under stable or acute conditions. There was no statistical difference in Hill's 'constant "n" among all groups studied.

Correlations were examined. With all acute and stable asthmatic patients *pooled*, a correlation was found between $P_{50_{7.40}}$ and pH ($r=0.6$, $p<0.001$) and $P_{50_{7.40}}$ and HCO_3- ($r=0.31$, $p<0.02$). No correlation was observed between $P_{50_{7.40}}$ and $PaCO_2$, PaO_2, SaO_2, hemoglobin or 2,3-DPG. Analysis of *stable* state asthma revealed correlations of $P_{50_{7.40}}$ with pH ($r = 0.4$, $P<0.02$) and HCO_3- ($r = 0.5$, $p<0.02$) *only*, while in *acute* asthma $P_{50_{7.40}}$ correlated only with pH ($r = 0.6$, $p<0.01$).

Any effect of therapeutic agents, including adrenal corticosteroids, upon $P_{50_{7.40}}$ cannot be delineated from the present data, in view of reported rightward shifts associated with steroids.[12]

Erythrocytic Organic Phosphate Changes

The mean (± 1 SD) concentration of erythrocytic 2,3-DPG in the normal controls of 3.44 ± 0.79 $\mu M/10^{10}$ RBC was *not* significantly different from chronic stable or acute bronchial asthma with respective levels of 3.82 ± 1.32 and 3.17 ± 1.28 $\mu M/10^{10}$ RBC. Similarly, acute and stable asthmatics as a group exhibited no differences in 2,3-DPG values (Tables 1 and 2). Paired data analysis (Table 3) correspondingly revealed similar 2,3-DPG levels in acute asthma compared with the same patient in the stable state. Data for ATP paralleled these 2,3-DPG observations. Patients with COLD manifested significant increases in 2,3-DPG compared with normal controls ($P<0.05$) and acute asthmatics ($P=0.05$).

Correlation analysis of all asthmatics as a group revealed no relationship between $P_{50_{7.40}}$ and 2,3-DPG. There was a low ($r = 0.32$) relationship only between 2,3-DPG and arterial pH ($P<0.02$). When the asthmatic population was divided into either stable or acute groups, no correlation between 2,3-DPG and $P_{50_{7.40}}$, pH or other variables was observed.

Subdivision of Acute Bronchial Asthma into Hyperventilation and Hypoventilation Groups: Reanalysis of Parameters

Based upon a spectrum of ventilatory changes, the *acute* asthmatic patients were arbitrarily divided into two groups defined by their effective alveolar ventilation as indexed by $PaCO_2$: *Hyperventilation* when $PaCO_2 \leq 37.0$ mm Hg, and *hypoventilation* (and cross-over phase) when

Table 4—*Subdivision of Acute Bronchial Asthma by Effective Alveolar Ventilation ($PaCO_2$): Comparison of Parameters.*

	Hyperventilation $PaCO_2 < 37.0$ mm Hg	Hypoventilation $PaCO_2 > 37.1$ mm Hg	Observations No.	Differences
No.	7	6	—	—
Age, yr	35.3 ± 15.0	44.2 ± 15.4	15	NS
PaO_2 mm Hg	59.6 ± 9.5	48.9 ± 8.0	13	NS
SaO_2, %	89.5 ± 4.9	80.2 ± 8.1	13	S, p <0.02
$PaCO_2$, mm Hg	31.2 ± 6.4	43.8 ± 5.4	15	S, p <0.01
pH	7.43 ± 0.07	7.35 ± 0.04	15	S, p <0.01
HCO_3^-, mEq/L	20.1 ± 3.8	23.3 ± 1.9	15	NS
Hgb, gm %	15.1 ± 1.9	14.7 ± 1.7	15	NS
2,3-DPG, $\mu M/10^{10}$ RBC	3.60 ± 1.80	2.76 ± 0.58	15	NS
ATP, $\mu M/10^{10}$ RBC	0.92 ± 0.64	0.92 ± 0.64	15	NS
$P_{50_{7.40}}$, corrected CO	27.7 ± 4.3	25.1 ± 0.7	15	NS
$P_{50_{7.40}}$, uncorrected CO	27.0 ± 3.9	24.9 ± 0.7	15	NS
Duration, hr	41.1 ± 22.8	36.6 ± 20.5	15	NS
HBCO, %	2.7 ± 2.2	0.70 ± 0.82	14	NS
FVC, % predicted	45.0 ± 10.4	38.5 ± 19.1	5	NS
Severity, 1-4+*	2.7 ± 0.8	3.4 ± 0.9	15	NS
MCHC	33.20 ± 0.0	30.52 ± 0.0	8	NS

NS = Non-significant S = Significant difference, p given *=4+ most severe

$PaCO_2 \geq 37.1$ mm Hg.[13,14]

There were seven patients in the hyperventilation category and six with hypoventilation. Their mean ages, clinical severity, FVC (percent predicted), hemoglobin concentrations, percent HbCO and duration of symptoms were *not* statistically significantly different. PaO_2 tended to be lower in the hypoventilating group, but was not of statistical significance; SaO_2, however, was significantly lower. Mean $PaCO_2$ was higher ($P<0.01$) but this was due to arbitrary selection of cases. Corresponding pH was significantly more acid (7.35 ± 0.04) in the hypoventilating than the hyperventilating group ($pH = 7.43 \pm 0.07$) ($P<0.01$). *No* differences in $P_{50\,7.40}$, 2,3-DPG or ATP were found when these two groups were compared with each other (Table 4).

However, when the hyperventilating and hypoventilating groups were compared to chronic stable bronchial asthma or normal controls, differences were noted (Table 5). In the group with alveolar *hypoventilation*, the mean $P_{50\,7.40}$ (corrected CO) of 25.1 ± 0.7 mm Hg was significantly lower than in chronic bronchial asthma, $P_{50} = 27.6 \pm 1.6$ mm Hg ($P<0.001$), and normal controls, $P_{50} = 26.8 \pm 0.3$ mm Hg ($P<0.001$). Similarly, the 2,3-DPG concentration (2.76 ± 0.58 $\mu M/10^{10}$ RBC) was significantly reduced over chronic stable asthma and normal controls ($P<0.02$ and $P<0.02$). ATP changes were not significant. Finally, the arterial pH of 7.35 ± 0.04 was more acid than stable asthma ($P<0.001$) and normal controls ($P<0.001$). $PaCO_2$ was significantly higher than in normal controls and in chronic stable asthma; HCO_3- and hemoglobin were not significantly different.

Similar analysis for the subgroup of hyperventilating asthmatic subjects revealed *no* changes in $P_{50\,7.40}$ (corrected CO) or 2,3-DPG; while $PaCO_2$ was lower (by selection), pH and HCO_3- were not different from control normals or stable asthmatics. Other variables are presented in Table 5. Due to sample size limitations, correlation analyses were not performed in these subgroups.

Finally, the total group of asthmatic patients was arbitrarily divided into two groups according to PaO_2 (<55 and >55 mm Hg); no differences in $P_{50\,7.40}$, 2,3-DPG or other variables were noted.

MCHC measurements were not statistically different between control subjects and patient groups (Table 1).

Discussion

In the present study oxygen-hemoglobin affinity was examined in a group of asthmatic patients

Table 5—*Comparison of Acute Asthma Versus Normal Controls and Stable Bronchial Asthma.*

Parameter	N*	$PaCO_2<37.0$ mm Hg Acute Vs Stable Asthma	N*	Acute Vs Normal Controls
$P_{50\,7.40}$, corrected CO	32	NS	34	NS
PaO_2, mm Hg	32	S, $p<0.001$	34	S, $p<0.001$
SaO_2, %	32	S, $p<0.001$	34	S, $p<0.001$
pHa	31	NS	34	NS
$PaCO_2$, mm Hg	31	S, $p<0.02$	34	S, $p<0.001$
HCO_3^-, mEq/L	30	S, $p<0.01$	34	S, $p<0.02$
Hgb, gm %	32	S, $p<0.02$	34	NS
2,3-DPG, $\mu M/10^{10}$ RBC	31	NS	33	NS
ATP, $\mu M/10^{10}$ RBC	30	NS	32	NS
Age, yr	36	NS	34	NS
HbCO, %	32	NS	34	NS
P_{50}, uncorrected	34	NS	34	NS
Parameter	N*	$PaCO_2>37.1$ mm Hg Acute Vs Stable Asthma	N*	Acute Vs Normal Controls
$P_{50\,7.40}$, corrected	33	S, $p<0.001$	25	S, $p<0.001$
PaO_2, mm Hg	31	S, $p<0.001$	23	S, $p<0.001$
SaO_2, %	31	S, $p<0.001$	23	S, $p<0.001$
pHa	32	S, $p<0.001$	25	S, $p<0.001$
$PaCO_2$, mm Hg	32	S, $p<0.001$	25	S, $p<0.01$
HCO_3^-, m Eq/L	31	NS	25	NS
Hgb, gm %	33	NS	25	NS
2,3-DPG, $\mu M/10^{10}$ RBC	32	S, $p<0.02$	24	S, $p<0.02$
ATP, $\mu M/10^{10}$ RBC	33	NS	25	NS
Age, yr	35	NS	23	S, $p<0.01$
HbCO, %	32	S, $p<0.02$	23	S, $p<0.02$
P_{50}, uncorrected	35	S, $p<0.001$	25	S, $p<0.01$

*Number of observations.

exhibiting various degrees of hypoxemia and ventilatory competence. Under *chronic* stable state conditions of approximately 12 days' duration, modest impairment of oxygen transfer in association with hypocapnia and respiratory alkalosis was present; however, SaO_2 was normal. Under these circumstances neither shifts in oxyhemoglobin affinity by $P_{50\,7.40}$ nor changes in intraerythrocytic organophosphate were observed. Based upon reports in the literature, the levels of SaO_2 and pH for this group with stable bronchial asthma are not of sufficient magnitude to alter 2,3-DPG biosynthesis or otherwise influence $P_{50\,7.40}$.[15,16] The *in vivo* position of the oxygen-hemoglobin association-dissociation curve may, however, be somewhat left of normal in stable asthma due to a Bohr effect related to chronic respiratory alkalosis.[17]

The pathophysiologic conditions of an *acute* bronchial asthmatic attack are, however, complex and despite the observation of a mean severity of 3+ (out of a possible 4+) score, one cannot predict the status of oxygen delivery to the tissues. Criteria other than $PaCO_2$ and pH, such as FVC, PaO_2, SaO_2, age, etc, may be quite similar in hyperventilating or hypoventilating subjects with acute asthma.[13] Therefore, to pool all such patients as a group might obscure significant changes in $P_{50\,7.40}$ related largely to carbon dioxide and to pH. Thus, in the present study, $P_{50\,7.40}$ measurements in acute bronchial asthmatic patients compared with normal controls and chronic stable bronchial asthma were statistically similar suggesting no hemoglobin adaptation despite significant hypoxemia and oxyhemoglobin desaturation. In addition *mean* $PaCO_2$ and pH were normal and would exert no effect upon 2,3-DPG levels. This conclusion is based upon *group* data and supported by paired observations.

However, the clinical spectrum of an acute asthmatic attack encompasses not only variable degrees of progressive hypoxemia, but may also be separated into stages of appropriate alveolar hyperventilation and decompensated alveolar hypoventilation.[13,18] The latter may also include a "relative hypoventilation" with approximately normal $PaCO_2$ and pH values, the so-called "cross-over point," which, in the clinical context, reflects a deteriorating phase preceding frank hypercarbia and respiratory acidosis.[14] Accordingly, a group of acute asthmatic patients may represent a heterogeneous population in terms of carbon dioxide clearance (effective alveolar ventilation) and the net effect upon arterial pH. This is reflected in the data of this series by the normal $PaCO_2$ and pH of the grouped acute asthmatic subjects.

When patients with acute bronchial asthma were divided into those with alveolar hyperventilation or those with progressive or overt hypoventilation by both clinical and $PaCO_2$ indices, certain interesting features were identified. In the group of hyperventilating patients, significant hypoxemia with a PaO_2 of 59.6 ± 9.5 mm Hg was present in conjunction with hypocapnia and respiratory alkalosis. Despite the presence of these two factors, $P_{50\,7.40}$ remained unchanged; 2,3-DPG was similarly not increased in this group over normal controls or stable state asthmatics. The cause for this failure of response is unclear from our data in view of the reports of rightward P_{50} shifts under similar conditions in other diseases.[3,6,19] Other than significant hypocapnia and hypobasemia, the other factors examined in this study, including changes in red cell volume (MCHC), appeared not to be distinctive in these clinically compensated hyperventilating acute asthmatic patients.[9]

Those asthmatic patients with relative or frank hypoventilation were acidotic with significantly lower SaO_2 levels; nevertheless, they exhibited a significantly reduced $P_{50\,7.40}$ when compared to both chronic stable state asthma or normal control subjects ($P<0.001$). Correspondingly, erythrocytic 2,3-DPG levels were significantly reduced ($P<0.02$). Thus these patients not only failed to adapt their $P_{50\,7.40}$ but were *left-shifted* despite significant hypoxemia and oxyhemoglobin desaturation. This effect may be in part ascribed to the coexisting hypercapnia and respiratory acidosis. Similar pH related effects, paralleling this hypercapneic-respiratory acidotic suppression in acute asthma have been described: altitude induced increases in 2,3-DPG are prevented by concurrent administration of acidifying agents;[20] inspired carbon dioxide administered to hypoxic rats prevents increases in red cell 2,3-DPG;[15] anemic or anoxic patients with acidosis have low 2,3-DPG concentrations.[19,21] Thus these observations in acute and status asthma support the concept that the time average Hb-HbO_2 ratio is not the total nor dominant regulatory mechanism for P_{50} shifts and that plasma and intraerythrocytic[19] pH changes, and perhaps elevated carbon dioxide concentrations as well, exert, in a multifactorial interaction, significant control in oxygen-hemoglobin equilibria.

The only significant correlations observed in this study were between $P_{50\,7.40}$ and pH and between 2,3-DPG and pH. They are in agreement with the above cited effects of pH upon 2,3-DPG and P_{50}. Based upon the observations that the formation of carbamino compounds, due to increases in P_{CO_2},[22] elevate P_{50} of constant pH hemoglobin solutions,

effects of P_{CO_2} levels in asthma were examined. However, changes in $PaCO_2$ did not appear to be an influence *per se*, but the influence of carbon dioxide on these data cannot be fully assessed. MCHC observations between normals, stable and acute asthmatics were not significantly different over the time course of this study. Thus changes in red cell volume did not influence the oxygen-hemoglobin affinity relationships described here.[10]

Investigations of most other patient groups with hypoxic challenges, and in particular those with pulmonary disorders have generally revealed an adaptation by rightward shifts of the oxyhemoglobin dissociation curve. Thus on ascent to altitude, in the several series of obstructive lung disease, including that of Lenfant et al, the latter characterized by severe hypoxemia (mean $PaO_2 = 47.5 \pm 8.7$ mm Hg) and chronic stable hypercapnia (51.1 ± 7.3 mm Hg) in patients with elevated hematocrits ($\geq$ 50 percent), all exhibit either *increases* in P_{50} or 2,3-DPG.[1,2,4,17] That such shifts in P_{50} can occur with milder degrees of hypoxemia in patients with pulmonary granulomatosis and fibrosis is described.[6] Finally, our COLD control patients revealed significant increases in P_{50} while the asthmatic subjects did not, despite similar degrees of hypoxemia.

The failure of our acute asthmatic population to respond to significant hypoxemia is only partially explicable. Hypoxemic, hyperventilating patients with bronchial asthma manifest conditions favoring both a rise in 2,3-DPG and a rightward shift in P_{50}. Yet no such shift was evident. The discrepancy remains unclear and the implication of this finding in the context of a serious or potentially lethal asthmatic attack is speculative. It may well bear clinical relevance that asthmatic patients under such acute conditions suffer from more serious tissue hypoxia than is evident from usual blood gas or clinical parameters, and until proved otherwise should be guarded against "dangerous" levels of hypoxemia. This problem appears to be further compounded by actual *leftward shifts* in P_{50} that appear partially pH dependent in hypoventilating asthmatic patients; this may further limit oxygen delivery and add to the burden of cardiorespiratory work. These statements must, however, be qualified by the influence of the direct Bohr effect, which may offset pH related 2,3-DPG suppression and result in *more physiologic in vivo* oxygen-hemoglobin relationships. When the mean *in vivo* P_{50} was determined for the group of hypoventilating asthmatic patients, a value for $P_{50\,7.35}$ (corrected for CO) of 26.2 mm Hg was found indicating that a rightward shifting tendency was present, partially compensating for the acidosis in contrast to the P_{50} determined at pH 7.40 of 25.1 mm Hg.[9] Conversely, this argument may be applied to hyperventilating asthmatics who would, for example from the data of this study, shift leftwards from $P_{50} = 27.7$ mm Hg at pH 7.40 to $P_{50} = 26.8$ mm Hg at a pH of 7.43. However, the critical experiments of actual tissue delivery of oxygen are yet to be performed to further clarify these changes in oxygen releasing capacity in bronchial asthma within the context of cardiovascular, red cell mass and ventilatory mechanisms.

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