The Effect of Cardiac Disease on Hemoglobin-Oxygen Binding

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ABSTRACT The relation between degree of cardiac functional impairment and changes in hemoglobin-oxygen affinity and 2,3-diphosphoglycerate (2,3-DPG) has been studied in 39 patients with noncyanotic heart disease. A progressive decline in hemoglobin-oxygen affinity was found with worsening cardiac function as assessed by cardiac index, arteriovenous oxygen (A-V O_2) difference, and cardiac symptoms; this alteration in hemoglobin-oxygen binding represents a significant mechanism for adaptation to the limited oxygen supply imposed by the cardiac lesion. The highly significant correlation of mixed venous blood oxygen saturation ($S\bar{v}_{O_2}$) with 2,3-DPG and the position of the oxygen dissociation curve suggests that the level of deoxygenated hemoglobin is an important in vivo regulator of hemoglobin-oxygen affinity.

INTRODUCTION

Heart disease, by reducing the capacity to pump blood, limits the amount of oxygen available to tissues. The result is hypoxia unless other mechanisms compensate for the reduced blood flow. One such mechanism is a decrease in the oxygen affinity of hemoglobin, mediated by an increase in erythrocyte 2,3-diphosphoglycerate (2,3-DPG). This mechanism has been shown to participate in the adaptation to high altitude (1) and to anemia.[1] Studies by Morse, Cassels, and Holder (2) and by Metcalfe, Dhindsa, Edwards, and Mourdjinis (3) indicate that changes in the oxygen dissociation curve occur with cardiac disease. The following study was undertaken to define the nature and extent of such changes in a group of patients with varying degrees of cardiac dysfunction.

METHODS

39 patients with clinical evidence of cardiac disease admitted to the hospital for diagnostic cardiac catheterization were studied. None of the patients had congenital heart disease of the cyanotic type. Specific diagnoses, based on findings at catheterization, and functional status according to the New York Heart Association criteria (4) are listed in Table I.

Measurements of hemoglobin concentration and oxygen affinity, as well as erythrocyte 2,3-DPG and adenosine triphosphate (ATP), were made on a single blood sample usually taken at the time of or within a few days of cardiac catheterization. Hemoglobin was measured as cyanmethemoglobin. The oxygen-hemoglobin dissociation curve was determined by the mixing technique (5). The results were expressed as the P_{50} (7.4), which is the partial pressure of oxygen in millimeters of mercury required for 50% saturation of hemoglobin at pH 7.4. Red cell organic phosphate compounds were separated by ion-exchange chromatography

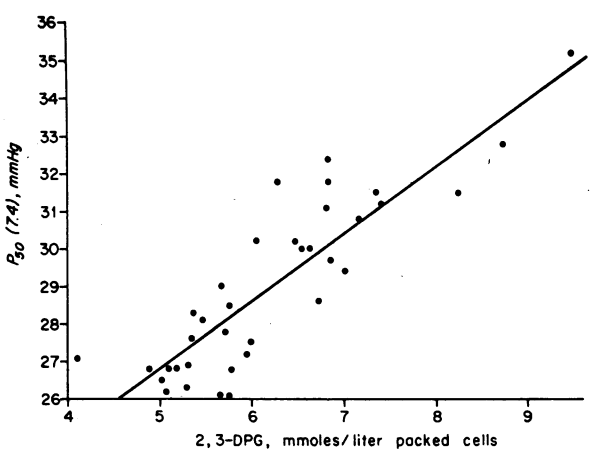

FIGURE 1 Relation between hemoglobin affinity for oxygen expressed by P_{50}, and erythrocyte 2,3-DPG in 39 cardiac patients. The equation of the regression line is $P_{50} = 1.80$ DPG + 17.86 (SEE = 1.1, $r = 0.87$, $P < 0.0001$).

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[1] Torrance, J., P. Jacobs, A. Restrepo, J. Eschbach, C. Lenfant, and C. A. Finch. Intraerythrocytic adaption to anemia. *N. Engl. J. Med.* In press.

TABLE I

Clinical Details and Collected Data in 39 Patients with Cardiac Disease

Patient No.	Age	Sex	Diagnosis	Functional class*	Tobacco usage	Hb	2,3-DPG	ATP	P_{50} (7.4)	AV O_2 difference	Cardiac index	Hb flow	$S\bar{v}_{O_2}$†
	yr				*cigarettes/day*	*g/100 ml*	*mmoles/liter*	*mmoles/liter*	*mm Hg*	*vol.%*	*liters/min/m²*	*g/min/m²*	*%*
1	56	M	Calcific aortic stenosis, aortic regurgitation	3	0	11.5	6.86	1.59	29.7	6.2	2.8	347	59
2	60	F	Rheumatic heart disease, mitral stenosis	2	2	13.2	5.76	1.35	28.5	6.4	2.3	312	61
3	70	F	Rheumatic heart disease, mitral stenosis, mitral regurgitation	3	0	13.9	7.01	1.19	29.4	10.7	1.4	182	37
4	53	M	Arteriosclerotic heart disease	4	20	15.8	5.98	0.91		8.1	2.3	308	51
5	24	M	Rheumatic heart disease, aortic regurgitation	4	§a	10.4	6.82	1.29	32.4	5.7	3.0	298	52
6	27	F	Idiopathic pulmonary hypertension	3	0	12.8	5.30	1.21	26.3	5.9	3.0‖	363	55
7	47	M	Arteriosclerotic heart disease, mitral regurgitation	3	20	9.1	6.87	1.19		3.8	4.5	335	55
8	43	F	Atypical chest pain	2	10	13.8	5.19	0.86	26.8		2.8‖	330	
9	43	F	Rheumatic heart disease, mitral stenosis	3	0	15.7	6.04	1.42		6.5	1.8¶	283	65
10	25	F	Congenital heart disease, pulmonic stenosis	2	0	15.4	5.66	1.09	26.1	3.9	3.8	532	73
11	44	M	? arteriosclerotic heart disease, mitral regurgitation	1	20	15.2	5.07	1.27	26.2	5.1	2.6	384	70
12	30	F	Congenital heart disease, atrial septal defect	2	20	14.9	4.11	1.02	27.2	3.5	4.4	620	80
13	40	F	Atypical chest pain	1	30	14.9	6.63	1.19	30.0	4.4	3.1	384	72
14	35	F	Congenital heart disease, atrial septal defect, anomalous pulm. venous drainage	2	0	14.4	5.76	1.24	26.1	5.7	2.3	325	77
15	24	F	Congenital heart disease, ventricular septal defect, ruptured sinus of Valsalva	3	0	12.7	5.99	1.01	27.5	4.1	3.4	443	77
16	33	M	Congenital heart disease, atrial septal defect	1	0	16.5	5.10	1.15	26.8	4.2	3.5	519	75
17	58	F	Rheumatic heart disease, mitral stenosis	3	0	14.2	5.71	1.29	27.8	6.8	2.7	365	56
18	56	F	Rheumatic heart disease, mitral stenosis	3	0	13.5	5.35	0.78	27.6	3.9	2.6	326	72
19	46	M	Left atrial myxoma	3	0	12.8	8.24	1.06	31.5	8.0	1.5	195	48
20	43	M	Congenital heart disease, situs inversus, corrected transposition, mitral regurgitation	3	25	11.9	7.40	1.51	31.2	7.4	2.3	290	52
21	42	F	Rheumatic heart disease, mitral stenosis	3	15	11.0	6.73	1.13	28.6	4.5	2.9	297	63
22	65	M	Congenital heart disease, atrial septal defect	3	0	16.2	5.95	1.04	27.2	4.7	2.8	408	73
23	44	F	Arteriosclerotic heart disease, rheumatic heart disease, mitral regurgitation, aortic stenosis, aortic regurgitation	2	15	13.0	6.47	1.27	30.2	4.4	2.4	302	70
24	55	F	Rheumatic heart disease, mitral regurgitation	3	0	12.3	8.73	1.33	32.8	10.2	1.3	160	33
25	20	M	Rheumatic heart disease, mitral stenosis	2	?	9.4	7.34	1.46	31.5	5.6	3.7	339	50
26	45	F	Rheumatic heart disease, mitral stenosis	2	20	14.5	7.16	1.10	30.8	8.4	1.3	164	46
27	34	F	Congenital heart dis., atrial septal defect, anomalous pulm. venous drainage	3	15	17.0	5.31	0.93	26.9		2.9‖	452	

TABLE I—(*Continued*)

Patient No.	Age	Sex	Diagnosis	Functional class*	Tobacco usage	Hb	2,3-DPG	ATP	P_{50} (7.4)	AVO_2 difference	Cardiac index	Hb flow	$S\bar{v}O_2$‡
	yr				*cigarettes/day*	*g/100 ml*	*mmoles/liter*	*mmoles/liter*	*mm Hg*	*vol.%*	*liters/min/m²*	*g/min/m²*	*%*
28	52	F	Rheumatic heart disease, mitral stenosis	3	0	16.3	6.05	1.98	30.2		2.4‖	328	
29	68	M	Arteriosclerotic heart disease, mitral regurgitation	4	0	13.7	6.83	1.25	31.8	9.2	1.4	200	46
30	62	M	Rheumatic heart disease, mitral stenosis	3	0	13.0	5.46	1.10	28.1	4.9	2.2	257	61
31	52	F	Hypertensive CV disease, mitral regurgitation	2	20	13.2	5.78	1.09	26.8	4.6	3.2	404	69
32	34	F	Rheumatic heart disease, mitral regurgitation, mitral stenosis	3	0	13.2	6.81	1.23	31.1	4.4**	3.5	432	69
33	21	F	Congenital heart disease, atrial septal defect	1	0	14.8	5.02	1.14	26.5	4.8	3.4	473	70
34	64	F	Rheumatic heart disease, mitral stenosis	1	15	13.1	5.67	0.59	29.0	5.3	2.6	333	65
35	64	M	Calcific aortic stenosis	2	0	14.6	6.28	0.74	31.8	6.0	3.6	480	62
36	59	M	Calcific aortic stenosis	3	0	11.9	6.54	0.65	30.0	5.9	2.5	295	58
37	49	M	Mitral regurgitation	3	0	12.7	9.43	1.14	35.2	9.7	1.4	179	36
38	34	M	Rheumatic heart disease, aortic regurgitation	2	§b	15.0	4.89	0.77	26.8	5.5	2.8	421	68
39	45	M	Calcific aortic stenosis	2	§c	15.3	5.37	0.99	28.3	6.0**	2.4	338	64

* According to the New York Heart Association Criteria (5).
‡ Derived in those patients breathing oxygen (see text).
§ a: chews tobacco; b: smokes pipe, cigars; c: smokes six cigars.
‖ Measured by biplane angiography.
¶ Measured by dye dilution.
** Based on measurement of blood from right ventricle.

TABLE II

*Hemoglobin, 2,3-DPG, ATP, and P_{50} in Cardiac Patients and Normal Controls**

	No. of patients studied	Hb ±1 SD	DPG ±1 SD	ATP ±1 SD	P_{50} ±1 SD
		g/100 ml	*mmoles/liter*	*mmoles/liter*	*mm Hg*
Cardiac patients					
Males	17	13.24 ±2.25	6.50 ±1.17	1.12 ±0.26	29.9 ±2.5
Females	22	13.99 ±1.36	6.01 ±0.92	1.16 ±0.26	28.4 ±1.9
Smokers‡	13	13.65 ±2.04	6.03 ±0.92	1.09 ±0.22	28.5 ±1.7
Nonsmokers	21	13.91 ±1.45	6.39 ±1.15	1.17 ±0.28	29.2 ±2.5
All patients	39	13.66 ±1.84	6.22 ±1.06	1.14 ±0.26	29.0 ±2.3
Normal controls					
Nonsmokers					
Males	20	15.32 ±1.02	4.83 ±0.33	0.87 ±0.20	27.1 ±0.8
Females	20	12.68 ±0.96	5.28 ±0.40	0.80 ±0.17	27.5 ±0.9
Total	40	14.00 ±1.65	5.06 ±0.44	0.83 ±0.19	27.3 ±0.9
Smokers‡					
Males	10	15.50 ±0.94	5.14 ±0.59	0.89 ±0.19	27.0 ±1.3

* Controls were obtained from reference 2.
‡ Smokers defined as those who smoked 10 cigarettes or more per day.

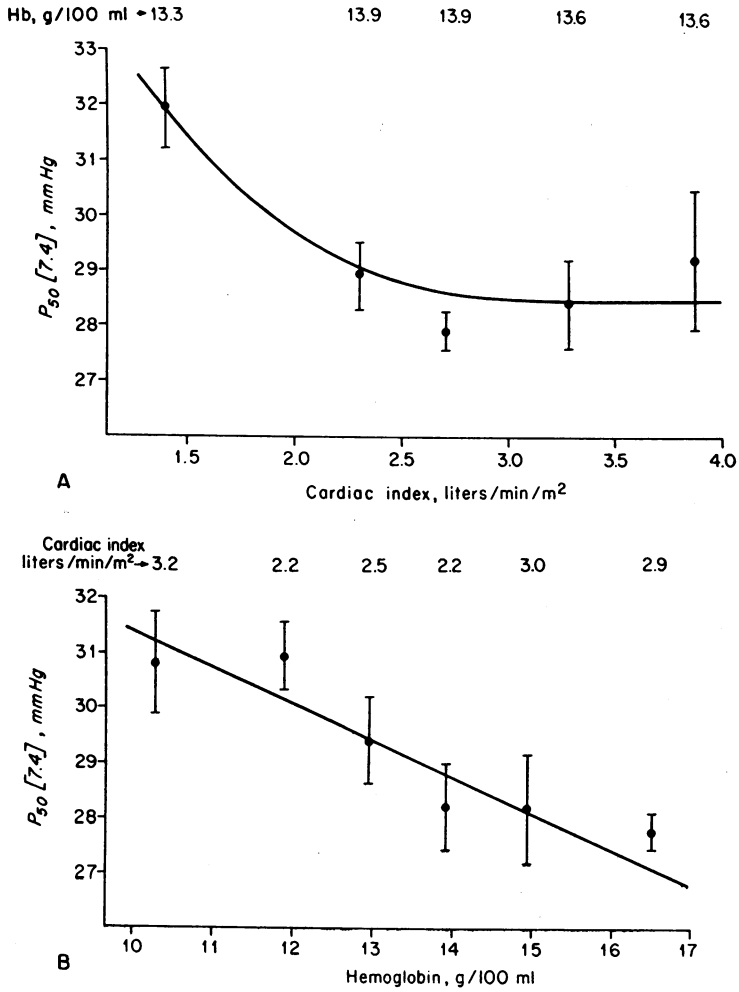

FIGURE 2 Relation between P_{50} and cardiac index (A) and P_{50} and hemoglobin concentration (B). Points are mean P_{50} ±1 SE for given intervals of cardiac index (0.5 liter/min per m^2) and hemoglobin (1.0 g/100 ml), respectively. Curve for P_{50} vs. hemoglobin (B) is regression of the individual observations used to calculate the above means ($P_{50} = -0.66$ Hb + 38.0, SEE = 2.0, $r = -0.49$, $P < 0.005$). There are no significant differences in the hemoglobin concentrations shown for each interval of cardiac index (A), nor in cardiac indices for each interval of hemoglobin concentration (B).

of a trichloroacetic acid extract using a modification[1] of the method of Robinson, Loder, and deGruchy (6). Total phosphorus was measured by the method of Bartlett (7). The 0.2 N ammonium chloride fraction was taken to represent 2,3-DPG and the 0.5 N ammonium chloride fraction to represent ATP.

Cardiac output was determined by the Fick method in 34 of the 39 patients. Of the remaining five, none of whom had mitral or aortic regurgitation, cardiac output was calculated in four from biplane angiograms (8) and in one by the dye dilution technique. For determining cardiac output by the Fick method, oxygen content of systemic arterial blood and pulmonary arterial blood ($C\bar{v}_{O_2}$) was measured manometrically by the Van Slyke technique. In two patients the pulmonary artery could not be entered and it was necessary to analyze blood from the right ventricle. In the seven patients with left-to-right intracardiac shunts, mixed venous oxygen content was approximated from the oxygen content of blood taken from the superior and inferior vena cava according to the expression:

$$C\bar{v}_{O_2} = \tfrac{1}{3}Csvc_{O_2} + \tfrac{2}{3}Civc_{O_2}.$$

Mixed venous blood oxygen saturation ($S\bar{v}_{O_2}$) was obtained from the ratio of $C\bar{v}_{O_2}$ to oxygen capacity. Oxygen consumption was measured by the closed circuit method using a Collins spirometer in 20 patients breathing oxygen and by the open circuit method in 14 patients breathing air.

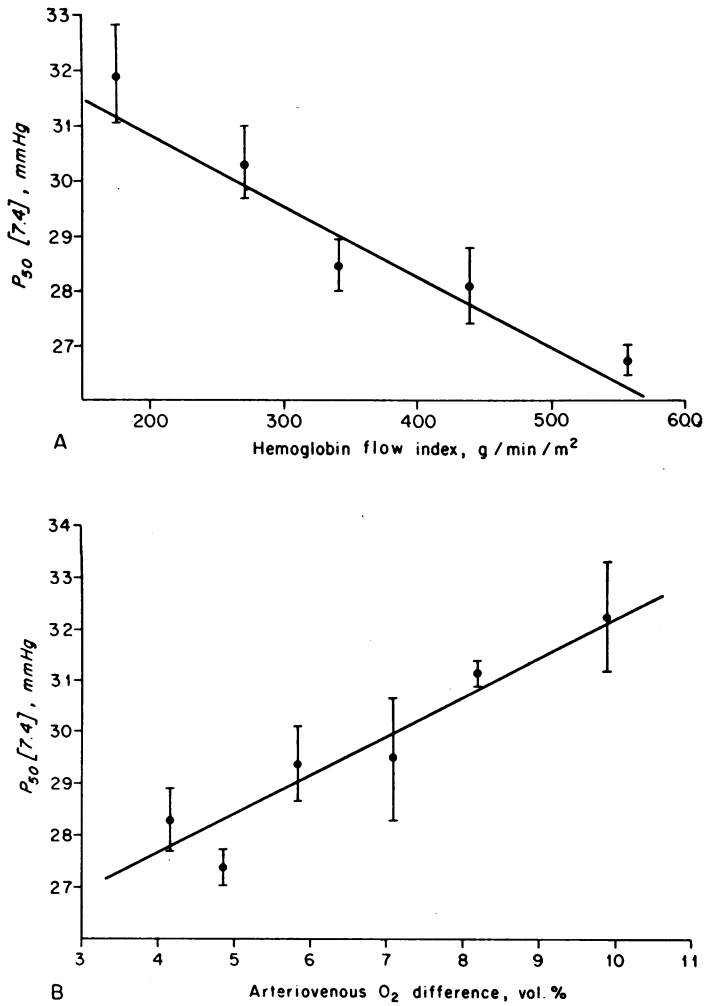

FIGURE 3 Relation between P_{50} and hemoglobin flow index (A) and P_{50} and A-V O_2 difference (B). Points are the mean P_{50} ±1 SE for given intervals of hemoglobin flow index (100 g/min per m²) and A-V O_2 difference (1.0 vol %). Curves are regressions of the individual observations used to calculate the above means ($P_{50} = -0.013$ Hb flow index + 33.43, SEE = 1.9, $r = -0.60$, $P < 0.0001$), and $P_{50} = 0.75$ AV + 24.64, SEE = 1.8, $r = 0.61$, $P < 0.0001$, respectively.

Expired volume was measured in a Tissot spirometer and gas concentration analysis was performed by the Scholander microtechnique.

A derived value for hemoglobin concentration was calculated by dividing the blood oxygen capacity, after allowance for dissolved oxygen (9), by 1.34. This value was slightly lower (mean $\Delta = 0.69$ g/100 ml) than that measured by cyanmethemoglobin, a difference which may be a consequence of inactive pigment. This hemoglobin value was subsequently used in the calculation of the hemoglobin flow index, defined as cardiac index times hemoglobin concentration.

Healthy laboratory and professional personnel, comprising both nonsmokers and smokers, served as controls.[1]

RESULTS

Table I details the findings in each of the patients studied. Mean cardiac index and arteriovenous oxygen (A-V O_2) difference ±1 SD in the 39 cardiac patients were 2.7 ±0.8 liters/min per m² and 6.0 ±1.9 vol % respectively. Oxygen consumption was essentially normal at 146 ±25 ml/min per m². The mean P_{50} (7.4) was 29.0 ±2.3 mm Hg, the mean 2,3-DPG was 6.22 ±1.06 mmoles/liter and the mean ATP was 1.14 ±0.26 mmoles/liter, all of which were significantly increased ($P < 0.001$) compared to the mean normal values of 27.3 ±0.9, 5.06 ±0.44, and 0.83 ±0.19, respec-

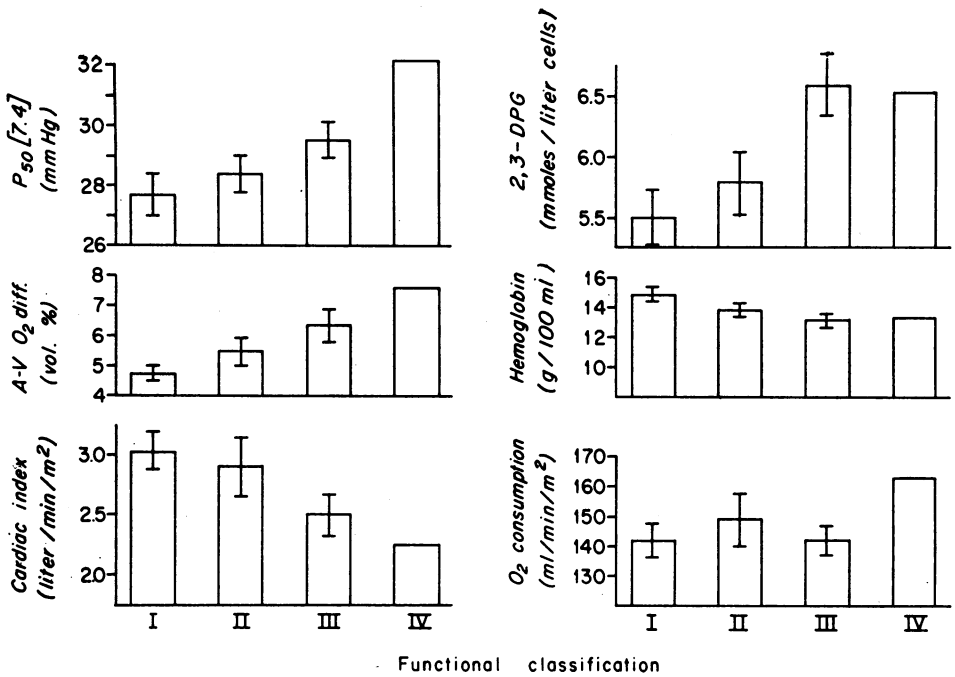

FIGURE 4 Cardiac index, arteriovenous oxygen difference, P_{50}, 2,3-DPG, hemoglobin, and oxygen consumption ±1 SE by functional classification of patients. Classes I, II, III, and IV contain 5, 12, 19, and 3 patients, respectively.

tively[1] (Table II). The relationship between P_{50} and 2,3-DPG in cardiac patients is shown in Fig. 1. There was a slight increase in ATP with increasing 2,3-DPG ($r = 0.28$, $P < 0.05$), but no statistically significant relationship was found between ATP and P_{50}. Similarly, the correlation between organic phosphate and the position of the oxygen dissociation curve was not improved by relating the sum of 2,3-DPG and ATP to P_{50}. Thus ATP is excluded from further relationships.

Hemoglobin-oxygen affinity was found to be related to several of the interdependent physiological factors which determine delivery of oxygen to cells. First, hemoglobin-oxygen affinity was observed to decrease with decreasing blood flow. While there was little or no change in P_{50} with mild reductions of blood flow (Fig. 2 A) a sharp increase was observed ($P < 0.001$) when cardiac index fell below 2 liters/min per m^2. Hemoglobin-oxygen affinity was also affected by the hemoglobin level; Fig. 2 B shows a progressive fall in affinity as the hemoglobin level decreases ($r = -0.49$, $P < 0.005$). Because both blood flow and hemoglobin concentration influence oxygen transport, one would anticipate a close correlation between P_{50} and oxygen flow to tissue. As is shown in Fig. 3 A, such a relationship between P_{50} and hemoglobin flow index was found ($r = -0.60$, $P < 0.0001$). Fig. 3 B depicts the relationship between P_{50} and the A-V O_2 difference ($r = 0.61$, $P < 0.0001$). All these relationships were independent of the smoking habits of the patients.

The results of the various measurements are portrayed in Fig. 4 in relation to the degree of functional disability (4). As might be expected, there was a relationship between symptoms and worsening cardiac function, as indicated both by the A-V O_2 difference and the cardiac index. Hemoglobin concentration while varying from patient to patient bore no apparent relationship to the clinical disability of the patient or to changes in output. Oxygen consumption also did not relate to the severity of the disease, with the possible exception of the three patients in class IV. With increasing disability, there were progressive increases in 2,3-DPG and P_{50}.

DISCUSSION

Many types of cardiac disease have the common feature of limiting cardiac output with a resultant decrease in oxygen flow to tissue. However, the obligatory tissue oxygen consumption, reflected by the normal oxygen consumption in the patients studied, indicates the presence of some compensatory adjustment. In patients with cyanotic congenital heart disease, this compensation is at least partially provided by a marked increase in hemoglobin which permits more oxygen transport per unit of blood flow. In the present group, however, a similar increase in hemoglobin concentration would

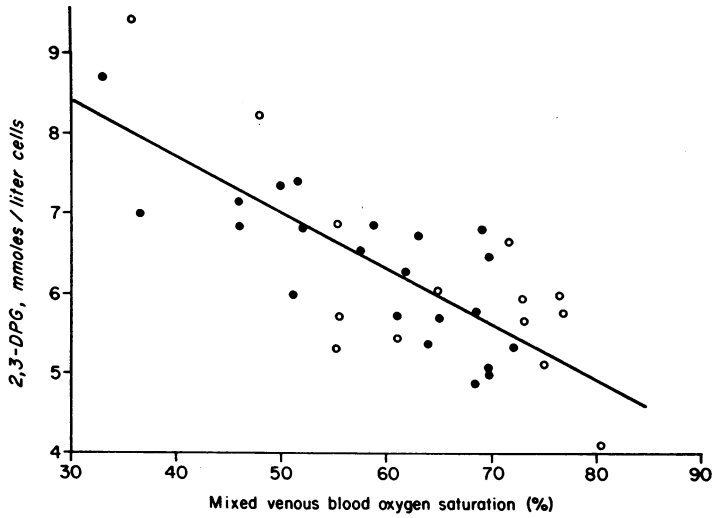

FIGURE 5 Relationship between 2,3-DPG and mixed venous blood oxygen saturation ($S\bar{v}_{O_2}$). Values of $S\bar{v}_{O_2}$ in patients breathing oxygen (closed circles) have been corrected (see text) to make them comparable to those of patients breathing air (open circles). Equation for regression is DPG $= -0.069$ $S\bar{v}_{O_2} + 10.48$ (SEE $= 0.71$, $r = -0.76$, $P < 0.0001$). Note that normal $S\bar{v}_{O_2}$ (75%) and 2,3-DPG (5.06 mmoles/liter) fall approximaately on the constructed line.

create a greater blood viscosity and an unacceptable increase in cardiac work. The alternative involves increasing the amount of oxygen extracted per circulation of the blood; such increased extraction is facilitated by a reduction in hemoglobin-oxygen affinity which partially maintains capillary oxygen tension and thus the diffusion gradient for oxygen. The present demonstration of a correlation between degree of cardiac abnormality and extent of changes in oxygen dissociation underscores the adaptive significance of these changes.

Recent studies have shown a mechanism capable of modifying oxygen release from hemoglobin in vitro (10, 11) which appears also to account for changes in oxygen dissociation observed in intact cells (1 and footnote 1). Under conditions of decreased oxygen availability, the glycolytic system of the red cell generates an increased amount of 2,3-DPG (12) which interacts with hemoglobin and decreases its affinity for oxygen. It has been suggested that the stimulus for the increased red cell glycolysis and 2,3-DPG rise is an increased amount of deoxygenated hemoglobin in venous blood. Since a widening of the A-V O_2 difference is an early feature of cardiac failure, we have analyzed the relation between the 2,3-DPG level and the $S\bar{v}_{O_2}$.[3] Fig. 5 shows the highly significant correlation between $S\bar{v}_{O_2}$ and 2,3-DPG ($r = 0.76$, $P < 0.0001$). A similar correlation ($r = 0.73$, $P < 0.0001$) was found between $S\bar{v}_{O_2}$ and P_{50}. Thus, as shown by the respective coefficients of correlation, adjustments in hemoglobin-oxygen binding correlate better with the mixed venous oxygen saturation than with cardiac index, hemoglobin, or hemoglobin flow index, all of which relate to tissue oxygen flow; likewise correlation with $S\bar{v}_{O_2}$ is better than with A-V O_2 difference, which relates to quantity of hemoglobin that is deoxygenated in the capillaries but not the proportion of oxygenated to deoxygenated hemoglobin. This correlation of $S\bar{v}_{O_2}$ with 2,3-DPG and P_{50} thus provides strong additional evidence that the level of deoxygenated hemoglobin must indeed be one of the main physiological factors which govern the position of the oxygen dissociation curve.

A change in proportion of oxygenated and deoxygenated hemoglobin might exert its influence on oxygen dissociation in two interrelated ways. First, 2,3-DPG synthesis might be stimulated by increased binding of this molecule by deoxygenated hemoglobin, which is the relatively greater (13, 14) or sole (15) binder of 2,3-DPG. Second, since deoxygenated hemoglobin is less acid than oxygenated hemoglobin, a change in 2,3-DPG synthesis and oxygen dissociation might result from a relative alkalosis in the red cell related to the fall in hemoglobin saturation.

Although the correlation of $S\bar{v}_{O_2}$ with 2,3-DPG and P_{50} is high, there is sufficient deviation from an ab-

[3] In patients breathing oxygen during cardiac output measurements, a $C\bar{v}_{O_2}$ was estimated by subtracting the observed A-V O_2 difference from that oxygen content corresponding to an assumed arterial saturation of 95%; this value was then converted to per cent saturation using the measured oxygen capacity.

solute relationship to suggest the influence of other factors. First, although pH was not measured in our study, many of the patients were taking diuretic agents, from which a rise in blood pH would be anticipated (16). The fact that pH affects the magnitude of the response of 2,3-DPG and P_{50} to impaired oxygen transport has recently been shown.[1] Second, there is the possibility that equilibrium may not have been achieved between circulatory status, which was measured at rest, and the position of the oxygen dissociation curve. It is well established that changes in 2,3-DPG and oxygen dissociation occur over a period of hours (1, 17), whereas circulatory adjustments, with attendant alteration in hemoglobin saturation, may occur instantaneously. Thus 2,3-DPG and P_{50} may correlate better with the average $S\bar{v}_{O_2}$ of the preceding several hours than with the isolated resting measurement. Finally, smoking would be expected to shift the oxygen dissociation curve to the left. However, as shown by Torrance et al.,[1] changes due to smoking in normal subjects were small compared to the changes associated with heart disease.

The quantitative significance of a shift of the oxygen dissociation curve can be analyzed by comparing the data from patients with severe cardiac dysfunction with those of normal subjects. In the six patients with the lowest cardiac outputs, the mean cardiac index was 1.39 liters/min per m^2; arteriovenous oxygen difference was 9.4 vol %; oxygen consumption was normal (131 ml/min per m^2). In these patients, because of the shift in the oxygen dissociation curve (mean $P_{50} = 31.9$ mm Hg), an oxygen extraction of 9.4 volumes per cent yields a mixed venous P_{O_2} of 30 mm Hg compared with the normal of 40 mm Hg. By contrast, had the curve not shifted, the mixed venous P_{O_2} would have been 25 mm Hg. While such a change appears to be of some protective significance, mixed venous P_{O_2} is, nevertheless, still far from normal. It is probable that additional compensation is effected by a redistribution of blood flow, with preferential shunting of blood from tissues which normally extract relatively little oxygen, such as the kidney and skin, to those which extract more (18, 19). While the final result of the compensatory mechanisms is maintenance of a normal resting oxygen consumption, the capacity of oxygen delivery to increase in response to further metabolic demand, as with exercise, is undoubtedly limited.

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