

Long-Term Studies of Hemoglobin-Oxygen Affinity in Hypoxemic Dogs with a Right-to-Left Cardiac Shunt¹

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Long-standing hypoxemia was surgically created in dogs by inserting an aortic homograft between the inferior vena cava and right atrium. Ligation of the caval-atrial junction resulted in a right-to-left cardiac shunt. Arterial pO_2 fell immediately and P_{50} increased within 20 min. The 2,3-diphosphoglycerate concentration rose in 4 hr following surgery, while hemoglobin concentration increased within 7 days. Alterations in hemoglobin-oxygen affinity can occur rapidly and may be beneficial compensatory responses to acute and chronic hypoxemia caused by a right-to-left cardiac shunt.

INTRODUCTION

Chronic hypoxemia secondary to cyanotic congenital heart disease is associated with a decreased hemoglobin-oxygen affinity [14]. Acute hypoxemia in human subjects taken to high altitude is also associated with a decrease in hemoglobin-oxygen affinity [9]. These properties suggest that facilitation of O_2 delivery is due to enhanced release of oxygen from the red blood cell [14].

We have described previously an animal model with hypoxemia similar to that found in patients with cyanotic heart disease [10]. This has allowed us to study immediate and long-term compensatory responses to and sequelae of blood oxygen deficiency. We wish to now report acute and chronic alterations in oxygen transport including changes in hemoglobin-oxygen affinity which occur in dogs with hypoxemia due to a substantial right-to-left cardiac shunt.

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METHODS

Fourteen dogs (14-24 kg) were made hypoxemic by surgically interposing an aortic homograft between the inferior vena cava and left atrium [10]. The inferior vena caval-right atrial junction was ligated and this resulted in a substantial right-to-left cardiac shunt. Blood samples were taken prior to and at regular intervals after surgery. Hemoglobin (Hb) concentration, arterial pO_2 , pCO_2 , and pH measurements were performed using standard laboratory procedures.

Oxyhemoglobin dissociation curves of whole blood were performed by the method of Duvelleroy *et al.* [6], and expressed as P_{50} (the partial pressure of oxygen at 50% saturation) corrected to a pH of 7.4.

2,3-Diphosphoglycerate (2,3-DPG) concentrations were determined enzymatically using Sigma kits 35 UV (Sigma Chemical Co., St. Louis, Mo.). In five animals blood total phosphate was measured and in two animals lactate concentrations were determined using an enzymatic determination by Sigma.

Control animals. Four dogs underwent a sham operation including all aspects of the procedure except insertion of the graft and ligation of the inferior vena cava. Blood samples were taken and procedures were performed as described for the hypoxemic animals.

Statistical evaluation of data was performed using Student's *t* test. Values are means \pm standard deviation.

RESULTS

I. Oxyhemoglobin Dissociation

All animals had normal P_{50} values of 27.1 ± 0.7 mm Hg before the onset of hypoxemia. The P_{50} increased as soon as 20 min after surgical completion of the shunt and continued to increase reaching a value of 31.7 ± 1.8 mm Hg by 4 hr postoperative (Fig. 1, Table 1). By 48 hr postshunt a maximum P_{50} of 32.8 ± 2.8 mm Hg was reached. It then stabilized and remained elevated during the remainder of the observation period (Figs. 1 and 2, Table 1).

II. 2,3-Diphosphoglycerate

2,3-DPG concentration was normal (0.93 ± 0.29 M/M Hb) in all animals before surgery and remained stable for 2 to 3 hr after completion of the shunt. Sharp in-

creases in 2,3-DPG concentration then occurred and these increases were significant ($P < 0.005$) by 4 hr postoperatively (Fig. 1, Table 1). Concentrations increased further and a maximum of 1.87 ± 0.33 M/M Hb was reached 48 hr after the onset of hypoxemia. 2,3-DPG concentration then decreased slowly during the following 9 to 12 months to 1.53 ± 0.13 M/M Hb and remained at this level (Figs. 1 and 2, Table 1) during the remainder of the observation period.

III. Arterial Blood Gases

Arterial pO_2 decreased as soon as the surgical shunt was completed, and the hypoxemia persisted throughout the period of the study (Fig. 3, Table 1). pO_2 rose slightly in the late follow-up period (Table 1). Whole blood pH decreased immediately after the onset of hypoxemia ($P < 0.3$) (Fig. 3, Table 1), returned to normal by 11–17 hr and remained constant for the remainder of the experiment (Table 1). The pCO_2 rose slightly after surgery but returned to normal after 4-hr and remained at this level throughout the experiment.

IV. Phosphate and Lactate

Total phosphate increased in five animals studied within 24 hr after the onset of hypoxemia (Table 2). This increase was significant ($P < 0.02$) at 7 days postshunt and remained so during the next 4 weeks when measurements were taken (Table 1).

Serum lactate concentration did not change in two animals studied during the first 3 weeks of hypoxemia.

V. Hemoglobin

Hemoglobin concentration increased within 7 days postoperative and continued to rise during the ensuing 2 months (Fig. 2, Table 1). It stabilized at 18.0 ± 1.0 g/100 ml ($P < 0.02$) within 2 to 3 months after surgery and remained elevated during the remainder of the experiment.

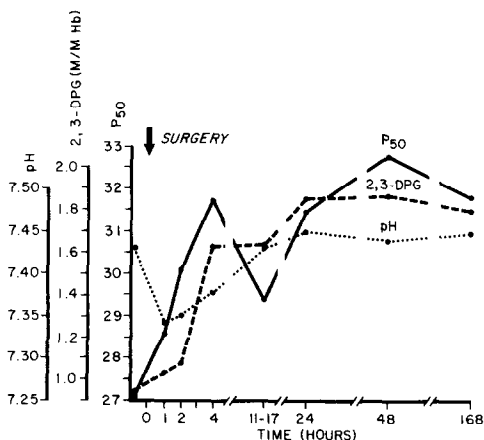


FIG. 1. Acute changes in blood P_{50} , 2,3-diphosphoglycerate (DPG), and arterial pH after surgical creation of a right-to-left cardiac shunt.

TABLE 1
BLOOD VALUES IN 14 SHUNTED DOGS, $FiO_2 = 21\%$

	Presurgery	Postsurgery (hr)						
		1	2 ^a	4 ^a	11-17 ^a	24 ^b	48 ^b	168 ^b
P_{50} 7.4 (mm Hg)	27.1 ± 0.7	28.6 ± 1.9	30.1 ± 0.5	31.7 ± 1.8	29.4 ± 1.1	31.4 ± 2.0	32.8 ± 2.8	31.8 ± 1.2
2,3-DPG (M/M Hg)	0.93 ± 0.18	1.03 ± 0.21	1.08 ± 0.30	1.64 ± 0.39	1.62 ± 0.23	1.86 ± 0.33	1.87 ± 0.33	1.79 ± 0.28
Hb (g%)	14.0 ± 1.6	—	—	—	—	—	—	—
pO_2 (mm Hg) ^f	83 ± 10	44 ± 6	47 ± 6	36 ± 3	36 ± 6	39 ± 4	40 ± 5	40 ± 4
pCO_2 (mm Hg) ^f	25.0 ± 5.0	36.5 ± 9.2	28.3 ± 2.3	26.6 ± 3.6	24.2 ± 4.8	24.0 ± 6.4	24.3 ± 5.0	22 ± 6
pH ^f	7.43 ± 0.06	7.34 ± 0.10	7.35 ± 0.05	7.38 ± 0.06	7.43 ± 0.05	7.45 ± 0.06	7.44 ± 0.03	7.45 ± 0.04

	Postsurgery (month)				
	1 ^c	2 ^d	3 ^e	12 ^e	48 ^e
P_{50} 7.4 (mm Hg)	32.3 ± 2.0	32.0 ± 1.0	31.2 ± 0.9	32.0 ± 1.9	31.6 ± 0.7
2,3-DPG (M/M Hb)	1.75 ± 0.02	1.69 ± 0.27	1.61 ± 0.29	1.53 ± 0.13	1.53 ± 0.24
Hb (g%)	16.5 ± 2.2	18.0 ± 2.1	18.0 ± 1.0	18.4 ± 0.9	18.2 ± 1.4
pO_2 (mm Hg) ^f	38 ± 8	37 ± 4	47 ± 7	43 ± 5	48 ± 6
pCO_2 (mm Hg) ^f	23 ± 3	27 ± 6	27 ± 2	27 ± 8	27 ± 7
pH ^f	7.46 ± 0.06	7.46 ± 0.07	7.48 ± 0.03	7.47 ± 0.08	7.47 ± 0.06

^a 14 dogs.

^b 9 dogs.

^c 13 dogs.

^d 10 dogs.

^e 3 dogs.

^f Arterial blood.

VI. Control Animals

The control animals were followed for 4 weeks after the surgical procedure. There was no significant change in the parameters studied (Table 3).

DISCUSSION

In the present study we have observed several compensatory responses to surgically induced hypoxemia. These time-related changes are directed toward increased oxygen-carrying capacity of blood and may be classified as rapid, intermediate, or long term [5].

In our experiments, rapid compensatory responses included a decrease in blood pH with the onset of hypoxemia. Concomitant with this decreased pH was an increase in P_{50} occurring as soon as 20 min after the onset of right-to-left cardiac shunting. The physiological role of intracellular erythrocyte pH in altering hemoglobin-oxygen affinity has long been recognized (Bohr effect). Although intraerythrocyte pH was not recorded in our animals, a linear rela-

tionship does exist between intra- and extraerythrocyte pH as long as organic phosphate concentration remains normal [8]. A decrease of 0.10 pH unit accounts for an increase in P_{50} of 2.5 mm Hg [15]. Thus, the decrease in pH of 0.09 unit observed in our dogs in the first hour postshunt was sufficient to account for the increased P_{50} of 1.5 mm Hg. With the increase in P_{50} ,

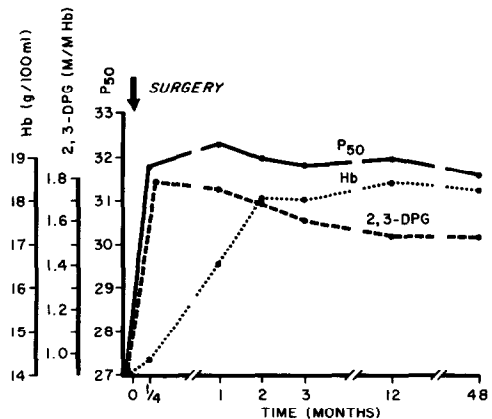


FIG. 2. Chronic changes in blood P_{50} , 2,3-diphosphoglycerate (DPG), and hemoglobin (Hb) after surgical creation of a right-to-left cardiac shunt.

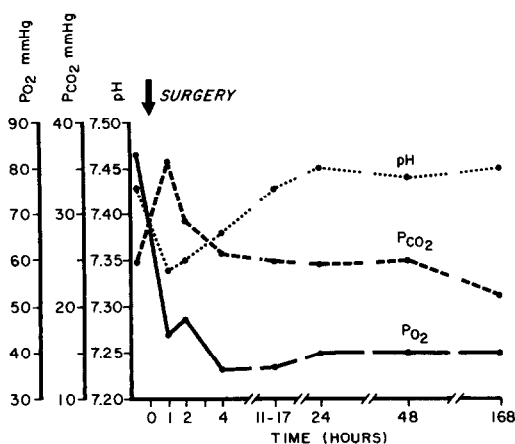


FIG. 3. Changes in arterial pO_2 , pCO_2 , and pH after surgical creation of a right-to-left cardiac shunt.

oxygen is theoretically released more readily by the red blood cell (RBC) at a tissue level.

A small but significant ($P < 0.01$) increase in pCO_2 also occurred during this time period. An increased CO_2 can result in an increased P_{50} , independent of pH, by binding to the N-terminal amino acids of the hemoglobin molecule [7].

The changes in pH and pCO_2 which we observed may be due to right-to-left cardiac shunting which was established acutely prior to a compensatory change in respiratory function. In these animals, the respiratory center may have been depressed by persistent effects of general anesthesia.

The intermediate responses to hypoxemia in our dogs include alterations in erythro-

cyte metabolism resulting in increased concentrations of 2,3-DPG. 2,3-DPG is the primary organic phosphate in the erythrocyte and has been shown to increase P_{50} by binding directly to the hemoglobin molecule [2, 4]. 2,3-DPG synthesis is pH dependent. A decreased intracellular pH decreases 2,3-DPG synthesis by reducing DPG mutase activity while increasing 2,3-DPG degradation by virtue of increased 2,3-DPG phosphatase activity [13].

The acidosis encountered in our experiments theoretically improved oxygen release by RBC for the first several hours while preventing increased 2,3-DPG synthesis. As soon as the pH returned to normal, 2,3-DPG synthesis increased, and this probably accounted for the continued elevation of the P_{50} (Fig. 2). 2,3-DPG synthesis remained stimulated and concentrations reached peak values 24–48 hr postoperative (Table 1). This elevated 2,3-DPG concentration remained throughout the experimental period although it later decreased slowly with a concomitant rise in hemoglobin concentration (Fig. 3). Concomitant with the increase in 2,3-DPG concentration, total whole blood phosphate levels increased 29% by 1 week postoperative. Phosphate levels remained elevated during the 4-week time period during which samples were taken. Serum lactate levels measured in two animals remained constant and at normal levels throughout the period of intermediate response to hypoxemia. This

TABLE 2

BLOOD PHOSPHATE AND LACTATE CONCENTRATIONS IN SHUNTED DOGS

	Presurgery	Post surgery				
		1 day	1 week	2 weeks	3 weeks	4 weeks
Total blood phosphate (mg%) ^a	3.2 ± 0.5	3.9 ± 1.1	4.5 ± 0.4	4.4 ± 0.4	4.6 ± 0.2	4.4 ± 0.2
Lactate (mM)						
dog No. 1	1.54	—	1.82	1.47	1.48	—
dog No. 2	1.40	—	1.43	1.42	1.36	—

^a 5 dogs.

TABLE 3
BLOOD VALUES IN FOUR SHAM DOGS, $FiO_2 = 21\%$

	Presurgery	Postsurgery			
		1-4 hr	24 hr	2 weeks	4 weeks
P_{50} (mm Hg)	27.1 ± 0.9	26.4 ± 2.1	26.7 ± 1.8	26.4 ± 1.8	26.7 ± 0.5
2,3-DPG (M/M Hb)	1.20 ± 0.06	1.23 ± 0.08	1.10 ± 0.16	1.14 ± 0.19	1.14 ± 0.25
Hb (g/100 ml)	12.8 ± 1.4	12.8 ± 1.4	12.9 ± 1.8	13.1 ± 1.4	13.1 ± 2.0
pO_2 (mm Hg) ^a	87 ± 8	82 ± 12	89 ± 8	82 ± 12	86 ± 11
pCO_2 (mm Hg) ^a	26 ± 2	28 ± 8	26 ± 3	25 ± 2	27 ± 3
pH ^a	7.41 ± 0.02	7.39 ± 0.02	7.40 ± 0.03	7.42 ± 0.01	7.43 ± 0.02

^a Arterial blood.

suggests no alteration in the animals' overall net metabolism.

Long-term adaptive responses to hypoxemia are largely hemopoietic with an increase in red blood cell mass [10]. Hb concentration in our animals was increased maximally at 2 to 3 months after surgery, and it remained stable and elevated after this. The etiology of this erythropoietic stimulation is clearly established, and the mechanism of its action involves increased erythropoietin production. When oxygen delivery is decreased to the renal cortical regulating center, erythropoietin production is stimulated. Erythropoietin acts directly on the bone marrow to stimulate RBC production [1]. With a significant rise in RBC mass and persistent elevation of P_{50} , oxygen delivery improves to the renal regulating center at a tissue level and erythropoietin production is reduced.

Other long-term adaptive responses to hypoxemia include an increase in capillary [3] and mitochondrial density [12]. Although the present study does not define these, studies are in progress to investigate these changes in dogs with a right-to-left cardiac shunt.

We have defined the morphology of the compensatory changes in oxygen transport when dogs are made hypoxemic with a right-to-left cardiac shunt. An understanding of these responses may allow manipulation of oxygen transport in order to enhance these compensatory mechanisms.

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