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Research Article

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Studies were also performed upon blood from a man with complete erythrocyte aplasia who received all of his red cells by transfusion from presumably normal persons. With mild anemia (hematocrit, 28%), the affinity of his blood for oxygen was slightly diminished (an oxygen pressure of 27.0 mm Hg was required to produce 50% saturation of hemoglobin with oxygen). With severe anemia (hematocrit, 13.5%), however, his blood had a markedly decreased oxygen affinity (an oxygen pressure of 29.6 mm Hg was required to produce 50% saturation of hemoglobin with oxygen).

We conclude that patients with various conditions characterized by an impairment in the oxygen supply system to tissues respond with a diminished affinity of their blood for oxygen. Although the mechanism which brings about this adaptation is not known, the displacement of the oxygen equilibrium curve is associated with an increase in heme-heme interaction. The decrease in blood oxygen affinity [...]

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Improved Oxygen Release: an Adaptation of Mature Red Cells to Hypoxia

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ABSTRACT Blood from patients with erythrocytosis secondary to arterial hypoxemia due either to congenital heart disease or to chronic obstructive pulmonary disease was shown to have a decreased affinity for oxygen; the average oxygen pressure required to produce 50% saturation of hemoglobin with oxygen was 29.8 mm Hg (average normal, 26.3 mm Hg). Such a displacement of the blood oxygen equilibrium curve promotes the release of oxygen from blood to the tissues.

Studies were also performed upon blood from a man with complete erythrocyte aplasia who received all of his red cells by transfusion from presumably normal persons. With mild anemia (hematocrit, 28%), the affinity of his blood for oxygen was slightly diminished (an oxygen pressure of 27.0 mm Hg was required to produce 50% saturation of hemoglobin with oxygen). With

severe anemia (hematocrit, 13.5%), however, his blood had a markedly decreased oxygen affinity (an oxygen pressure of 29.6 mm Hg was required to produce 50% saturation of hemoglobin with oxygen).

We conclude that patients with various conditions characterized by an impairment in the oxygen supply system to tissues respond with a diminished affinity of their blood for oxygen. Although the mechanism which brings about this adaptation is not known, the displacement of the oxygen equilibrium curve is associated with an increase in heme-heme interaction. The decrease in blood oxygen affinity need not occur during erythropoiesis, but may be imposed upon mature circulating red cells.

INTRODUCTION

Blood from patients with such diverse conditions as arterial hypoxemia due to congenital heart disease (1), high altitude exposure (2-4), and various types of anemia (5-10) has been shown to have a decreased affinity for oxygen. These etiologically and clinically dissimilar conditions have as a common physiological characteristic a decreased oxygen tension and/or concentration in arterial blood. The decrease in blood oxygen affinity can be thought of as a compensatory mechanism which, like erythrocytosis (11), or an increased rate of blood flow, tends to sustain the oxygen tension in blood perfusing tissue capillaries. To test this hypothesis, we measured the oxygen affinity of blood from patients with erythro-

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cytosis secondary to arterial hypoxemia produced by chronic obstructive pulmonary disease, a group not previously studied in this respect. Previous studies on blood from patients with cyanotic congenital heart disease were repeated with new improved techniques.

To clarify the mechanism by which a decrease in the oxygen affinity of blood is brought about in these patients, we calculated the slope of the blood oxygen equilibrium curve using Hill's n (12) as a measure of heme-heme interaction. We also studied blood from a patient with anemia due to complete erythrocyte aplasia. This man received all of his red cells from presumably normal donors by transfusion. We reasoned that if oxygen affinity of blood from this patient was decreased, it would indicate that the mechanism which alters blood oxygen affinity in conditions with an increased danger of tissue hypoxia is capable of acting upon circulating mature red cells.

METHODS

Patients were considered to have arterial hypoxemia if the oxygen pressure in arterial blood (P_{aO_2}) at rest was below 60 mm Hg, or if the oxyhemoglobin saturation in arterial blood (S_{aO_2}) was below 85%. Patients were considered to have erythrocytosis if their blood had a hematocrit greater than 55%, a hemoglobin concentration greater than 18 g/100 ml, or if the red cell mass was greater than 35 ml/kg of body weight. Patients were excluded if recent therapy (multiple phlebotomies or discontinuance of oxygen therapy) might have acutely altered their erythrokinetics.

Data from these patients were compared with those obtained from blood of normal volunteers, at rest, and after 20 min of breathing 10% oxygen.

We also studied blood from a man (P. L.) of 62 yr who was well until August 1964 when he noted the onset of progressive fatigue, dyspnea, palpitation, and throbbing in the head. At that time, he was found to have a severe anemia and all of his symptoms abated with blood transfusion, only to recur repeatedly as the anemia returned and to be regularly relieved with adequate blood transfusion. A complete absence of red cell precursors was found on repeated bone marrow studies and no reticulocytes were ever seen on multiple peripheral blood examinations. He received testosterone-estradiol, prednisone, and adrenocorticotrophic hormone (ACTH) without beneficial effect. In 1965, a large benign thymoma was resected without remission of his erythrocyte aplasia. His 24-hr urine erythropoietin excretion was consistently found to be extremely high, in the range of 2000 U/day (normal, 2-8 U). Beginning in 1966, slowly progressive leukopenia and thrombocytopenia were noted. We studied the oxygen-hemoglobin equilibria of blood from this

patient on 12 January 1967, and again on 22 August 1967. The patient died suddenly in October 1967. Postmortem findings supported the diagnosis of complete erythrocyte aplasia.

For each study, blood was taken from a peripheral vein without stasis into a syringe containing heparin and sodium fluoride. Oxygen-hemoglobin equilibrium values were determined using the "mixing technique" (13) at a carbon dioxide pressure (P_{CO_2}) of 40 mm Hg and 37°C. At least two values of blood oxygen pressure and pH, one above and one below 50% saturation of hemoglobin with oxygen, were determined, and the observed values were corrected for dissolved oxygen (13) and to a standard plasma pH of 7.40 (14). All studies were completed within 2 hr of venipuncture. Oxygen pressures (P_{O_2}) at 50% saturation of hemoglobin with oxygen were determined graphically and will be designated as P_{50} . In some studies, complete oxygen-hemoglobin equilibrium curves were determined and values were calculated for Hill's n (12), which indicates the degree of inflection of the oxygen-hemoglobin equilibrium curve, using the method of least squares. Hill's n may be regarded as an expression of the degree of heme-heme interaction. The magnitude of the Bohr effect was also determined using the "mixing technique" (13) after tonometry of blood

TABLE I
Oxygen Pressures Found in Blood from Patients with Arterial Hypoxemia and Erythrocytosis after Tonometry and Mixing (13) to Produce the Desired Values of Oxyhemoglobin Saturation

Patient	Oxygen saturation of hemoglobin (%)				Hill's n	Bohr factor*	pH ₅₄ †
	21.5	46.0	54.0	80.5			
	Oxygen pressures						
	mm Hg						
R. Y.		27.3	30.7	46.2	3.02		7.308
J. D.	17.7	27.3	30.5	45.1	2.89		7.350
L. R.	19.3	29.8	32.0	47.5	3.02	-0.51	7.315
H. W.	19.0	30.1	33.2	50.9	2.76		7.420
A. S.	20.5	28.3	32.3	51.9	2.89		7.495
K. K.		26.4	29.9	45.4	2.94	-0.45	7.469
W. B.	17.2	28.7	29.8	44.9	2.81	-0.44	7.513
Mean‡	18.7	28.8	31.6	48.1	2.90		
SD					0.10		

Values have been corrected for dissolved oxygen and to a plasma pH of 7.40. Calculated values of Hill's n (12), the magnitude of the Bohr effect and the uncorrected plasma pH (at a carbon dioxide pressure of 40 mm Hg and 54% oxygen saturation of hemoglobin) are also given.

* Bohr factor = $\Delta \log P_{O_2} / \Delta pH$, at 46 and 54% saturation.
† pH₅₄, Plasma pH measured at approximately 54% oxygen saturation of hemoglobin before correction to a standard plasma pH of 7.40.

‡ Patients J. D., L. R., H. W., A. S., and W. B.

|| All patients (including R. Y. and K. K.).

at high and low P_{CO_2} , at 46 and 54% saturation of hemoglobin with oxygen.

RESULTS

The effect of changes in plasma pH upon the position of the oxygen-hemoglobin equilibrium curve (Bohr effect) in blood from patients with arterial hypoxemia and secondary erythrocytosis was within the normal range (see Tables I and II). Therefore, the Severinghaus nomogram (14) was used to correct observed values to a plasma pH of 7.40. When the P_{CO_2} of equilibrating gas was 40 mm Hg, alkaline values for plasma pH were found after tonometry of blood from patients with respiratory failure, probably because they had respiratory acidosis and renal retention of bicarbonate. In contrast, the same in vitro P_{CO_2} (40 mm Hg) produced acid plasma pH values in blood from patients with cyanotic congenital heart disease, presumably because they had respiratory alkalosis secondary to hyperventilation induced by hypoxemia. The oxygen-hemoglobin equilibrium curves of blood from these two groups of patients came to lie in essentially the same position whether

TABLE II

Oxygen Pressures Found in Blood from Normal Subjects after Tonometry and Mixing (13) to Produce the Desired Values of Oxyhemoglobin Saturation

Subject	Oxygen saturation of hemoglobin, %				Hill's <i>n</i>	Bohr factor*
	21.5	46.0	54.0	80.5		
<i>mm Hg</i>						
B. A.	14.0	24.8	28.1	41.9	2.44	
M. N.	16.9	25.3	28.1	43.9	2.84	
M. E.	14.8	24.6	27.7	41.9	2.58	
J. W.	15.1	24.7	27.6	43.1	2.59	
J. M.	16.2	25.4	27.6	41.7	2.87	-0.44
F. R.	15.8	24.7	27.4	41.2	2.83	-0.46
S. S.	13.9	23.4	27.0	41.8	2.45	-0.55
D. W.	14.3	24.7	26.7	42.2	2.50	
G. T.	16.0	25.9	28.4	45.3	2.61	
Mean	15.2	24.8	27.6	42.6	2.64	
SD	1.1	0.7	0.5	1.3	0.17	

Values have been corrected for dissolved oxygen and to a plasma pH of 7.40. Calculated values of Hill's *n* (12) and the magnitude of the Bohr effect are also given.

* Bohr factor, $\Delta \log P_{O_2}/\Delta pH$, at 46 and 54% saturation.

TABLE III

Summary of Pertinent Findings in Patients with Erythrocytosis Secondary to Arterial Hypoxemia

Parent	Age	Sex	SaO ₂	Pao ₂	Paco ₂	pH _a	TBV	RBC mass	Hct	Hb	Edema	Diagnosis and relevant clinical observations	P ₅₀
	<i>yr</i>		%	<i>mm Hg</i>					%				
H. C.	19	F	78.1							21.2	0	Tetralogy of Fallot and kyphoscoliosis	30.4
E. H.	22	F	79.1						58		0	Common atrium	29.5
B. H.	25	F	78.0						57		0	Eisenmenger's syndrome and kyphoscoliosis	30.5
R. Y.	28	M	81.3						71		0	Patent ductus arteriosus with reversed shunt	29.0
J. D.	23	M	81.6						65	21.2	0	Eisenmenger's syndrome and kyphoscoliosis	28.9
L. R.	26	F	79.0						65	20.0	0	Tricuspid atresia, IASD, and IVSD	30.9
A. S.	36	M		50	65		67.9	48.0	70		2+	Intrinsic asthma and/or CB, obesity	30.3
H. W.	55	M		33					63		4+	Severe COPD, myocardial infarction, cirrhosis	31.6
C. S.	65	M		45	55	7.40	99.1	56.0	58		4+	Severe COPD with heart failure	29.1
C. K.	60	M		41	52	7.34	75.3	50.3	60		2+	Intrinsic asthma and/or CB, obesity	28.1
O. W.	70	M		53	35		85.1	50.7	59		2+	Unexplained cardiomegaly with pulmonary edema	29.8
W. B.	60	M		45	81	7.32	80.4	57.5	71		0	Severe obesity, heart failure, CB	29.3
												Mean	29.8
												SD	1.0

SaO₂, oxygen saturation of hemoglobin; Pao₂, arterial blood oxygen pressure; Paco₂, arterial blood carbon dioxide pressure; pH_a, arterial blood pH; TBV, total blood volume in ml/kg of body weight; RBC mass, red blood cell mass in ml/kg body weight; Hct, hematocrit; Hb, blood hemoglobin concentration (g/100 ml); P₅₀, partial pressure of oxygen (mm Hg) at 50% saturation of hemoglobin, 37°C, and plasma pH = 7.40; IVSD, interventricular septal defect; IASD, interatrial septal defect; COPD, chronic obstructive pulmonary disease; CB, chronic bronchitis.

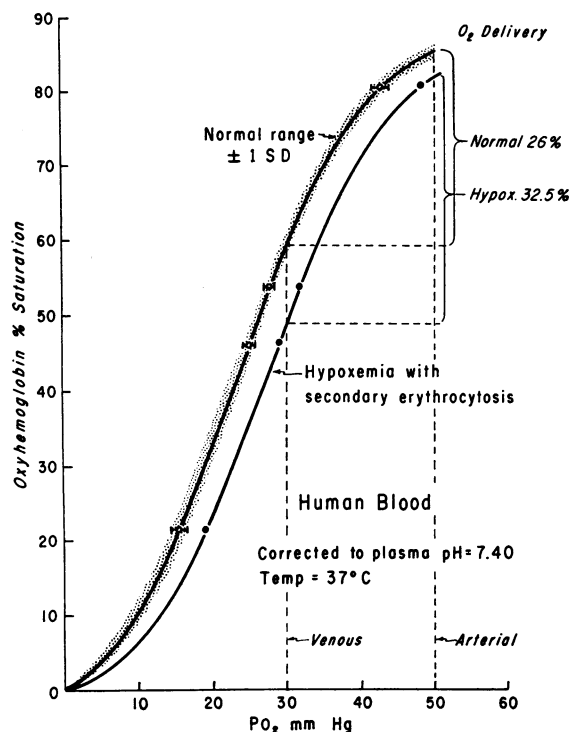


FIGURE 1 Oxygen-hemoglobin equilibrium curves of blood from normal subjects and from patients with hypoxic erythrocytosis.

the raw data were corrected from more alkaline or more acid plasma pH values to the standard pH of 7.40 (see Table I), further evidence that use of the Severinghaus nomogram was valid.

Blood from patients with arterial hypoxemia, due either to respiratory failure or to congenital vascular shunts, differed from normal (Table II) in showing an increased heme-heme interaction (increased Hill's n) ($P < 0.01$) (Table I) and a decreased oxygen affinity (increased P_{50}) ($P < 0.01$) (Table III). A composite oxygen-hemoglobin equilibrium curve for blood from patients with arterial hypoxemia is compared with the curve of blood from normal volunteers in Fig. 1.

Exposure of normal subjects to arterial hypoxemia (produced by breathing 10% oxygen for 20 min) did not change the position of the oxygen-hemoglobin equilibrium curve of blood from normal subjects (Table IV).

Values from two studies of blood from P. L. with total erythrocyte aplasia are given in Table V. A graphical presentation of his blood oxygen-hemoglobin equilibrium curve in comparison with

TABLE IV
Oxygen Pressures Found after Tonometry and Mixing (13) of Blood to Produce the Desired Values of Oxygen Saturation of Hemoglobin

Subject	Oxygen saturation of hemoglobin	Oxygen pressure		
		Composition of inhaled air		Change
		20.9% O ₂	10% O ₂	
	%	mm Hg		
C. W.	21.5	16.5	16.7	+0.2
M. E.	21.5	17.3	17.2	-0.1
J. M.	46.0	25.9	26.1	+0.2
C. W.	54.0	27.5	27.2	-0.3

Blood samples were drawn from normal subjects at rest (supine, breathing room air) and again at the end of a 20 min period of breathing 10% oxygen. Values have been corrected to a plasma pH of 7.40.

The duration and magnitude of arterial hypoxemia in each subject during 20 min of 10% oxygen inhalation is indicated by the following values of oxygen saturation of hemoglobin (obtained by ear oximetry): C. W.: below 80% last 8 min, ultimately 74%. M. E.: below 80% last 10 min, below 70% last 7 min, 60% last 5 min. J. M.: below 80% last 15 min, below 70% last 10 min, 66% last 3 min.

TABLE V
Oxygen Pressures Found after Tonometry and Mixing (13) of Blood to Produce the Desired Values of Oxyhemoglobin Saturation

I. 12 January 1967, hematocrit = 28%					
Oxygen saturation of hemoglobin, %					
	21.6	46.7	55.2	80.5	
Oxygen pressures, mm Hg					
	15.8	26.1	28.9	42.2	
	15.0	25.7	28.5	42.7	
Mean	15.4	25.9	28.7	42.5	
II. 22 August 1967, hematocrit = 13.5%					
Oxygen saturation of hemoglobin, %					
	23.2	49.1	57.3	82.3	
Oxygen pressure, mm Hg					Hill's n
	19.6	28.9	32.3	47.1	3.11
	19.1	29.6	32.5	47.7	2.98
	19.2	29.5	32.6	47.1	3.04
Mean	19.3	29.3	32.5	47.3	3.04
SD	0.2	0.4	0.2	0.3	0.07

Blood samples were drawn from P. L. on two different dates and at two different hematocrit levels. Values have been corrected for dissolved oxygen and to a plasma pH of 7.40. Calculated values of Hill's n (12) are also given.

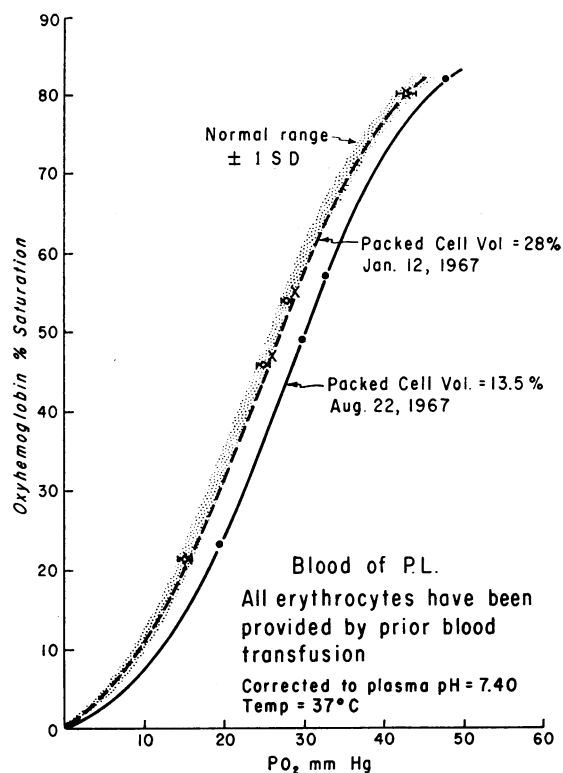


FIGURE 2 Oxygen-hemoglobin equilibrium curves of blood taken from P. L. at hematocrit levels of 28 and 13.5%, respectively, compared with normal values (stippled area).

that of normal blood is shown in Fig. 2. On January 12, when his hematocrit was 28% (his most recent transfusion had been given 6 days earlier), the oxygen affinity of his blood was virtually normal ($P_{50} = 27.0$ mm Hg). In contrast, on August 22, when his hematocrit was only 13.5% (the most recent transfusion had been given 15 days earlier), the oxygen affinity of his blood was markedly decreased ($P_{50} = 29.6$ mm Hg) and the degree of heme-heme interaction was also higher than normal (n of 3.04, normal $n = 2.64$). The increases in P_{50} and Hill's n in the August study were both statistically significant ($P < 0.01$) compared with normal values.

DISCUSSION

Blood from hypoxemic patients with compensatory erythrocytosis differs from normal blood in having a decreased oxygen affinity. This results in an improvement in its function of oxygen delivery to tissues. For example (as illustrated in Fig. 1),

with an arterial P_{O_2} of 50 mm Hg and a venous P_{O_2} of 30 mm Hg, blood from normal subjects will have an arterio-venous oxygen saturation difference of 26%, whereas blood from a patient with hypoxemic erythrocytosis will have an arterio-venous oxygen saturation difference of 32.5% with the same P_{O_2} values in arterial and venous blood. The decrease in oxygen affinity would thus provide 25% more oxygen to the tissues at the same rate of blood flow and the same blood hemoglobin concentration.

We have found a similar decrease in blood oxygen affinity in patients with cardiac failure (without erythrocytosis or arterial hypoxemia) and abnormally low values for cardiac output (15).

Hemoglobin from young red cells has a lower oxygen affinity and a greater heme-heme interaction than does hemoglobin from old red cells when the red cells from one individual are separated according to in vivo age (16). For this reason a recent change in the rate of erythropoiesis would be expected to affect the affinity of whole blood for oxygen by affecting the age distribution of circulating red cells. We therefore attempted to exclude from this study patients whose recent treatment might have caused an alteration in the rate of erythropoiesis.

Distinct from the effect of therapy, changes in the age distribution of circulating red cells probably do occur in the natural histories of hypoxemic patients and may, therefore, have contributed to our findings. The red cell life span has been reported to be normal in the erythrocytosis of high altitude (17), but somewhat shortened in that resulting from the hypoxemia of chronic obstructive pulmonary disease (18). Nucleated red cells have been found in significant numbers in the peripheral blood of patients with hypoxemia secondary to pulmonary disease and of patients with congestive heart failure (19). This suggests that exceptionally young red cells are being released into the circulation. In rats, erythropoietic stimulation is associated with marked reticulocytosis and marked shortening of red cell survival (20).

On the other hand, the oxygen affinity of mature circulating red cells is subject to alteration. In our patient with aplastic anemia (P. L.), all circulating red cells had undergone erythropoiesis and maturation in the bone marrows of other, presumably normal, individuals. Before transfusion, they had

undergone a 2–3 day period of storage at 4°C. Storage of blood has been shown to increase its oxygen affinity (we confirmed this finding in the blood subsequently given to P. L.), but the oxygen-hemoglobin equilibrium curve returns to normal within 24 hr after reinfusion (6). None of the red cells obtained from P. L. on 22 August 1967 could have been less than 15 days old and their mean age was probably older than those of a normal individual, an age bias which would tend to *increase* the oxygen affinity and *decrease* the heme-heme interaction of blood (16); our studies showed the opposite. This indicates that normal red cells, when transfused into an anemic person, can be modified in such a way as to decrease their oxygen affinity and to increase their heme-heme interaction. Comparison of the results of our two studies suggests that the degree of modification of oxygen affinity varied according to the severity of the anemia or to the length of time the foreign red cells were exposed to the tissues of this anemic patient. Apparently, the oxygen affinity of blood is not necessarily an intrinsic property of the red cells as they are synthesized in the bone marrow. We could not produce the change in blood oxygen affinity by producing arterial hypoxemia in normal subjects for 20 min.

We suggest that tissue hypoxia produces some alteration within the red cell which causes a change in hemoglobin function. A diffusible metabolite of hypoxic tissue might penetrate the red cell membrane to produce such an effect. A similar decrease in the oxygen affinity of blood occurs in patients with hepatic encephalopathy which can be transferred to normal red cells by *in vitro* incubation with a factor (or factors) separated from plasma by column chromatography (21). On the other hand, tissue hypoxia might act by extracting a greater percentage of the oxygen from the red cells. *In vitro* studies have shown that deoxygenated red cells have considerably greater glycolytic activity and thus produce more lactic acid and 2,3-diphosphoglycerate (DPG) than do red cells whose hemoglobin is combined with either of the ligands, oxygen or carbon monoxide (22). Either increased acidity (23, 24) or an increased concentration of DPG (25, 26) within the red cell would be expected to decrease hemoglobin's affinity for oxygen, although we currently have no evidence that either acidity (23, 24) or DPG (26, 27)

increase heme-heme interaction. DPG is readily bound to deoxygenated hemoglobin but does not bind to oxygenated hemoglobin (27). Benesch and Benesch have suggested that deoxygenation removes DPG from its free state, and thus stimulates glycolysis to replenish the stores of DPG and other organic phosphates (28), but whether tissue hypoxia is actually associated with increased red cell glycolysis is not known.

Regardless of its mechanism, a decrease in blood oxygen affinity may be a useful indicator of tissue hypoxia. We regard a decrease in blood oxygen affinity, like an increased blood hemoglobin concentration or an increased rate of blood flow, as another mechanism available to the organism for promoting the supply of oxygen to the tissues.

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