The Effect of Anemia on the Ventilatory Response to Transient and Steady-State Hypoxia

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ABSTRACT The effects of anemia upon the ventilatory responses to transient and steady-state hypoxia were studied in unanesthetized goats. Responses to transient hypoxia (inhalation of several breaths of nitrogen) were considered to reflect peripheral chemoreceptor-mediated reflexes whereas responses to steady-state hypoxia were considered to reflect both the chemoreceptor and non-chemoreceptor influences of hypoxia upon ventilatory control. In all goats, severe anemia (hemoglobin 3.1–4.8 g/100 ml) markedly heightened the responses to transient hypoxia (from a mean of 0.27 to a mean of 0.75 liter/min/percent fall in SaO_2). This phenomenon was substantially reversed by alpha-adrenergic blockade (phenoxybenzamine, 5 mg/kg). In contrast, the ventilatory responses to steady-state hypoxia were unaffected by severe anemia. These data suggest that severe anemia enhances the peripheral chemoreceptor-mediated response to hypoxia through a mechanism involving the alpha-adrenergic system. It also appears that a ventilatory depressant effect of hypoxia which is not mediated by the peripheral chemoreceptors is also enhanced by severe anemia, thereby preventing an increase in the steady-state ventilatory response to hypoxia. Finally, experiments involving variation in oxygen affinity of hemoglobin suggested that O_2 tension rather than O_2 availability in arterial blood is the major determinant of peripheral chemoreceptor activity.

INTRODUCTION

It has long been known that acute oxygen lack stimulates ventilation. However, many facets of this reflex are still obscure. It is not yet clear whether the peripheral arterial chemoreceptors sense oxygen tension or content of arterial blood (1). There is also considerable uncertainty concerning the effects of anemia upon ventilation per se and upon the ventilatory response to hypoxia (2, 3). This issue is of clinical interest since new modes of therapy for patients with hematologic malignancies and chronic renal failure have increased the prevalence of individuals with severe chronic anemia who are likely to encounter hypoxia-producing respiratory disorders.

One basis for the disparate results obtained in previous studies of the ventilatory response to hypoxia in anemia may be the use of steady-state methods. This reservation stems from the fact that ventilatory responses to all but exceedingly brief periods of hypoxia probably reflect the combined effects of stimulation of the peripheral chemoreceptors and of depression of ventilation via a direct effect upon the central nervous system (4). Anemia could complicate the situation by decreasing oxygen delivery to both the peripheral chemoreceptors and the brain.

The protocols of the present study were, therefore, designed to evaluate separately the peripheral chemoreceptor and the direct central nervous ventilatory effects of hypoxia in intact unanesthetized animals. In order to make this distinction, we took advantage of the fact that the peripheral chemoreceptor stimulation of ventilation occurs within 2–10 s after exposure to acute hypoxia (5), whereas the centrally mediated depression of ventilation by acute hypoxia takes 40–60 s to become manifest (6, 7). We, therefore, assessed the peripheral chemoreceptor responses to hypoxia by exposure of unanesthetized goats to brief inhalations of nitrogen and, in the same animals, we considered the responses to steady-state hypoxia to provide a measure of the combined effects of the peripheral chemoreceptor and central nervous effects of hypoxia.

From these experiments, we have been able to evalu-

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ate the roles of several factors in the overall ventilatory response to hypoxia during chronic anemia. These include oxygen content of arterial blood, the degree of affinity of hemoglobin for oxygen, the level of sympathetic nervous system activity, and the effects of hypoxia upon tissues other than the peripheral chemoreceptors.

METHODS

The experiments were performed on 17 goats which ranged in weight from 30 to 35 kg. 1 wk before the onset of the study, under general anesthesia, an indwelling arterial cannula was placed in the descending aorta via a carotid or femoral artery. During the week between the arterial cannulation and the experiments, the animals were trained to the test procedures by exposure to mock experiments and equipment in the laboratory. The animals were studied unanesthetized and were lightly restrained by fixing the horns to a post while standing upright.

The experimental procedure involved the use of a snug mask, with a dead space of 100 ml, fitted to a Rudolph one-way breathing valve (O. C. Rudolph and Sons Inc., Caldwell, N. J.). Wide-bore tubing at both ends of the valve made possible a switch from one inspired gas to another, or the collection of expired gas, without disturbing the goat. Ventilation was measured using a Fleisch pneumotachograph that was interposed in the inspiratory line and attached to a differential pressure transducer (Statham PM-15, Statham Instruments, Inc., Oxnard, Calif.). The pressure signal was converted to a signal proportional to tidal volume using an integrator circuit and records were made using an oscilloscopic recorder (Electronics for Medicine, Inc., White Plains, N. Y.). Calibrations of volume were made using a 2-liter syringe; these calibrations were repeated three times during each experiment. Each calibration deviated by less than 10% from the average value.

Transient hypoxia

The method for determining the ventilatory response to transient hypoxia was a modification of that originally described by Dejours (8). Before the start of the experimental procedure, the goats were anticoagulated with heparin (2 mg/kg) administered i.v. A valving system made it possible to admit either room air or nitrogen to the inspiratory end of the breathing valve without disturbing the goat. The test procedure consisted of allowing the goat to breathe varying quantities of nitrogen for brief periods (1–8 breaths per trial). Approximately 15 trials assured a wide range of levels of transient hypoxia. The oxygen saturation of arterial blood was monitored continuously during nitrogen breathing using a dual-wave length cuvette oximeter (Waters 0-500, Waters Associates Inc., Farmingham, Mass.). Blood was drawn from the arterial cannula through the cuvette by a motor-driven syringe (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 60 ml/min. The blood which was withdrawn during each period of nitrogen breathing (30–40 ml) was reinfused after the response to nitrogen inhalation had been recorded. The oximeter was calibrated using blood which had been drawn from the animal on the day of the experiment, equilibrated with high and low oxygen mixtures, and analyzed for oxygen saturation using the method of Van Slyke and Neill (9).

In two goats, a separate arterial catheter was used to record blood pressure. This allowed ventilation and blood pressure to be recorded simultaneously as blood was withdrawn. It was shown that removal of 30–40 ml as during the transient hypoxia studies was without effect on the ventilation, blood pressure, and heart rate. Separate studies comparing the effects of introducing the catheter via the carotid artery and the femoral artery also failed to disclose any difference in the observed data which might have been attributable to the different sites of cannulation.

Steady-state hypoxia and hypercapnia

The same valving system was used to deliver air followed by a mixture of 12% oxygen in nitrogen for 7 min each. Ventilation, blood pressure, and heart rate were recorded continuously. Arterial blood was collected anerobically after 6 min of breathing air and after 1.5 and 6 min of breathing the hypoxic mixture. Expired gas was collected for 1 min after 6 min of inhalation of each gas mixture. The blood samples were analyzed within 10 min using appropriate electrodes (Radiometer Co., Copenhagen, Denmark) at 37°C for P_{O_2}, P_{CO_2}, and pH. Oxygen saturation and hemoglobin content were determined by spectrophotometry (CO-Oximeter, Instrumentation Laboratory Inc., Lexington, Mass.). The volume of expired gas was measured with a calibrated dry-gas meter and samples were collected anerobically for determination of P_{O_2} and P_{CO_2} (Radiometer Co.). The accuracy of the analyzers was checked periodically using the micro-Scholander technique (10). Oxygen consumption, carbon dioxide production, and respiratory quotient were calculated in the usual way (11).

The ventilatory response to inspired CO_2 was determined by administering an inspired gas mixture of 5% CO_2 in 30% O_2 plus N_2 after a period of breathing a mixture of 30% O_2 in N_2. Each period lasted for 10 min. Expired gas and arterial blood were sampled during the last minute of each period. The volume of expired gas and the pH, P_{CO_2}, and P_{O_2} of the arterial blood were measured as above. The ventilatory response to CO_2 was expressed as the ratio of the increase in ventilation to the increase in arterial P_{CO_2}.

Groups of studies

Four groups of studies were done: (*a*) determination of the effects of anemia upon the ventilatory responses to transient hypoxia, (*b*) determination of the effects of alpha-adrenergic blockade upon the responses to transient hypoxia in normal and anemic animals, (*c*) determination of the effects of modification of O_2 availability at constant O_2 content of arterial blood upon the ventilatory responses to transient hypoxia, and (*d*) determination of the effects of anemia upon the ventilatory responses to steady-state hypoxia.

Effects of anemia on responses to transient hypoxia. Six goats were studied. Initial experiments were performed in duplicate at normal hemoglobin levels for the goat, i.e., 10–12 g/100 ml of blood. The goats were then bled to lower levels stepwise. No more than 500 cm^3 of blood were removed on any one day. Equivalent volumes of 0.9% NaCl were given as volume replacement. 3 days were allowed to elapse between the last bleeding and a study in order to ensure hemodynamic stability. Ventilatory studies were repeated in duplicate during moderate anemia (hemoglobin concentrations of 6–8 g/100 ml of blood) and severe anemia (2.5–4.5 g/100 ml of blood). Approximately 3 wk were required to complete a study of the ventilatory responses to transient hypoxia at these three ranges of hemoglobin concentration in any one goat.

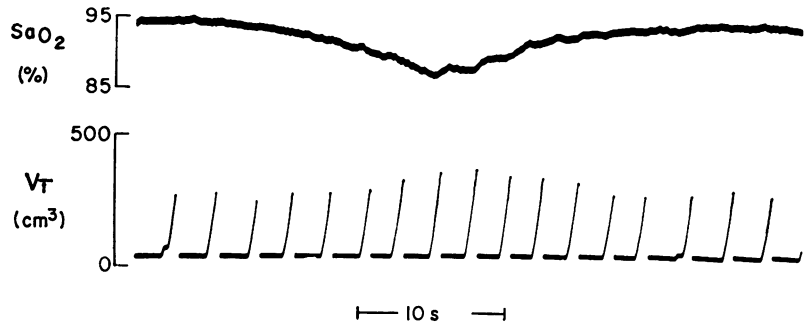

FIGURE 1 The ventilatory response to inhalation of three breaths of nitrogen. The fall in continuously monitored O_2 saturation (SaO_2) paralleled the rise in tidal volume (V_T) and respiratory rate.

Alpha-adrenergic blockade. Five additional goats were used in these studies. The ventilatory responses to transient hypoxia were determined in the control state as described above. On a subsequent day, phenoxybenzamine (5 mg/kg) was administered by i.v. infusion over a 1-h period. Arterial blood pressure was kept at near-control levels by infusion of dextran in 0.9% NaCl. The maximum decrease in mean blood pressure from control was 15 mm Hg. The ventilatory responses to transient hypoxia were then measured 3 h after the start of the infusion of the phenoxybenzamine. Persistence of alpha-adrenergic blockade at the end of the study was shown by the presence of a hypotensive effect of i.v. administered epinephrine (10 μg/kg). During the subsequent 12 days the goats were bled to hemoglobin levels of from 4 to 7 g/100 ml of blood. Ventilatory responses to transient hypoxia were then tested, as in the nonanemic goats, before and after the administration of phenoxybenzamine.

O_2 availability versus O_2 content of arterial blood. Six studies were done in five additional goats. A constant hemoglobin concentration (8 g/100 ml of blood) was maintained for each study while the O_2 affinity of hemoglobin was varied. The O_2 affinity was measured as the P_{O_2} required for 50% saturation of hemoglobin with O_2 (P_{50}).[1] Variations in the P_{50} were produced by taking advantage of the fact that, in the goat, the adaption of O_2 affinity of hemoglobin to anemia is a slow process, requiring 3–4 wk, which involves the synthesis of an electrophoretically distinct hemoglobin moiety (12, 13). Thus, different P_{50} levels were achieved at consistent levels of anemia (8 g/100 ml of blood) by a variety of maneuvers including rapid bleeding, slow bleeding, and recovery from severe anemia. Values for P_{50} were determined as described by Oski, Gottlieb, Miller, and Delivoria-Papadoupolous (14).

Steady-state studies. These studies were done on the same animals, and simultaneously with, the studies described in group 1. Two to four determinations were made of the ventilatory response to inhalation of 12% O_2 in N_2 at each of the three levels of hemoglobin concentration. Ventilatory responses to CO_2 were measured in these animals as well.

RESULTS

Effects of anemia upon the responses to transient hypoxia. Fig. 1 is a record obtained from a period of transient nitrogen inhalation. It illustrates that the peak ventilatory response coincided with the point of minimum oxygen saturation. The responses to transient hypoxia were, therefore, quantitated by relating the maximal increase in ventilation to the maximal fall in saturation for the periods of nitrogen exposure, as previously described (15). Maximal increase in ventilation was defined as the difference between the minute ventilation of the two consecutive breaths of greatest magnitude during nitrogen inhalation, and the average minute ventilation during the 25-s period immediately preceeding nitrogen breathing. Fig. 2 illustrates typical results obtained in one animal at three different levels of hemoglobin concentration. Each point represents the maximal increase in ventilation plotted against the maximal fall in oxygen saturation of arterial blood for a period of nitrogen inhalation. A linear relationship between the two variables was always observed. In each case, anemia increased the slope of the ventilation-SaO_2 relationship.

To illustrate the effects of anemia upon the ventilatory response to transient hypoxia for each animal, the slopes of the ventilation-SaO_2 line for each are plotted against hemoglobin level in Fig. 3. At normal hemoglobin levels the ventilatory responses to transient hypoxia ranged from 0.17 to 0.37 and averaged 0.27 liter/min/SaO_2 percent. In four studies the relationship between hemoglobin level and the ventilatory responses to transient hypoxia seemed hyperbolic such that reduction of hemoglobin level to 7–8 g/100 ml of blood had little effect, but further reduction to 6–7 g/100 ml of blood produced a consistent increase in response (average of 0.41 liter/min/SaO_2 percent). In the presence of severe anemia (2.5–4.5 g/100 ml of blood) even more striking increases to an average of 0.75 liter/min/SaO_2 percent were observed ($P < 0.005$). In two studies, the relationship between the responses to transient hypoxia and hemoglobin concentration seemed linear.

Effects of alpha-adrenergic blockade on responses to

[1] *Abbreviations used in this paper:* P_{50}, P_{O_2} required for 50% saturation of hemoglobin with O_2.

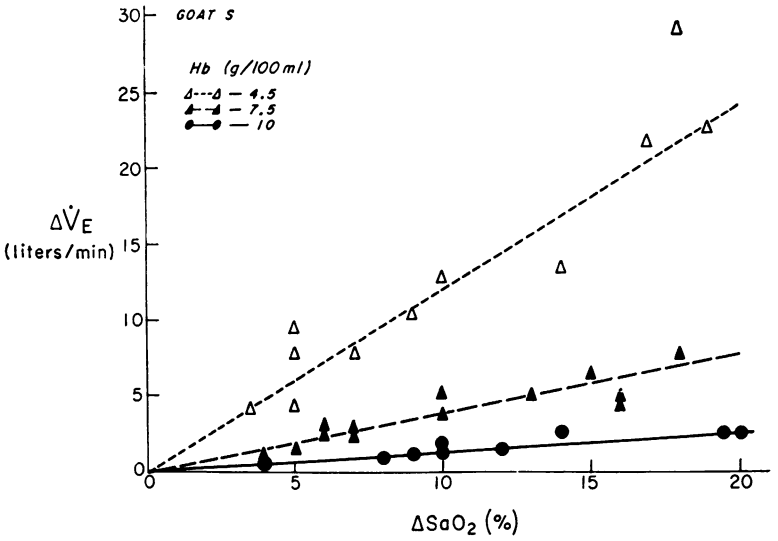

FIGURE 2 The effect of anemia upon the ventilatory response to transient hypoxia of one goat. Change in ventilation ($\Delta\dot{V}_E$) is plotted against change in SaO_2 for each period of nitrogen inhalation. The different symbols represent different concentrations of hemoglobin. The relationship between $\Delta\dot{V}_E$ and ΔSaO_2 was linear at all levels of hemoglobin concentration. Anemia enhanced the ventilatory response to transient hypoxia.

transient hypoxia. In a separate group of five goats, the effects of administration of phenoxybenzamine upon the ventilatory responses to transient hypoxia in the anemic and nonanemic state were studied. Fig. 4 illustrates the results. Alpha-adrenergic blockade virtually abolished the enhancement of transient responses which was associated with anemia but had no significant effect when the animals were not anemic.

Effects of variation of affinity of hemoglobin for O_2 upon responses to transient hypoxia. Fig. 5 shows the relationship between the ventilatory responses to transient hypoxia and P_{50} in five goats studied at a hemoglobin concentration of 8 g/100 ml of blood. The data suggest that there was a direct relationship between affinity of hemoglobin for oxygen and the responses to transient hypoxia. That is, a decreased P_{50} was associated with an increased responsiveness. In order to determine whether this effect could be explained by the peripheral chemo-

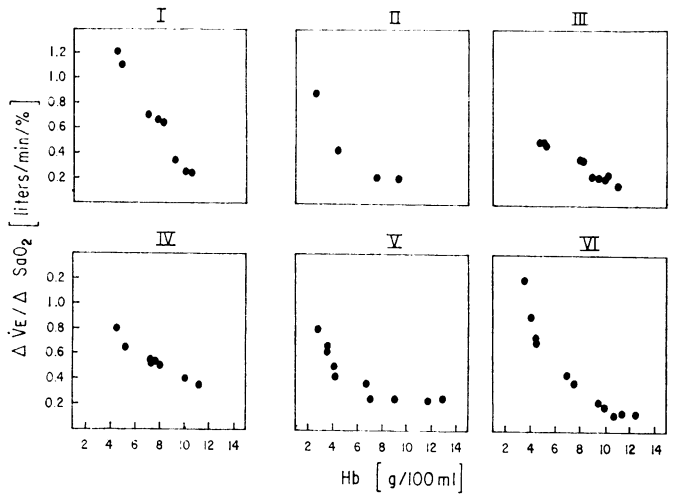

FIGURE 3 The effect of anemia upon the ventilatory response to transient hypoxia of six goats. The response to nitrogen inhalation expressed as the average increase in ventilation per unit fall in SaO_2 ($\Delta\dot{V}_E/\Delta SaO_2$), i.e. the slope of the lines shown in Fig. 2, is plotted against hemoglobin concentration. In each animal, anemia enhanced the response to transient hypoxia.

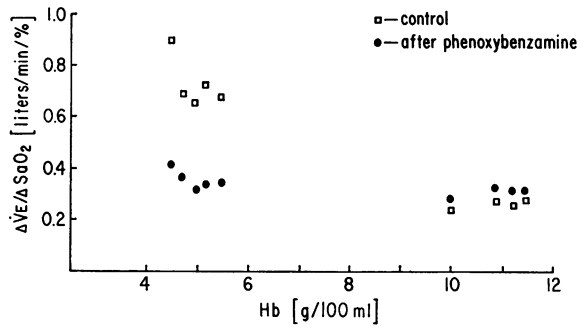

FIGURE 4 The effect of pretreatment with phenoxybenzamine (5 mg/kg) upon the ventilatory response to transient hypoxia (nitrogen inhalation) in anemic and nonanemic goats. The response to transient hypoxia is measured as in Fig. 3. The drug had virtually no effect in nonanemic animals but substantially reversed the heightened responses of anemic animals.

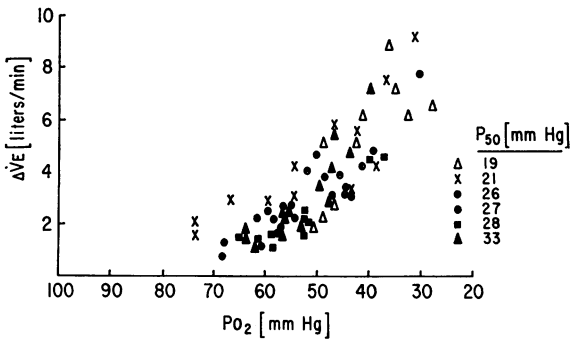

FIGURE 6 Individual data points which comprise the averaged data in Fig. 5 are replotted to illustrate the relationship between increases of ventilation ($\Delta\dot{V}_E$) and the minimum P_{O_2} for each period of N_2 inhalation. Although the goats had different affinities of hemoglobin for oxygen (P_{50}), a single relationship was found.

receptors' sensitivity to arterial O_2 tension rather than O_2 saturation, the data used to construct Fig. 5 were recalculated (using the O_2 dissociation curves determined for each study) so that ventilation could be plotted against arterial P_{O_2} for each period of nitrogen inhalation. This is shown in Fig. 6. The figure illustrates that despite differences in P_{50} levels, the response to transient hypoxia appeared to be a single function of arterial O_2 tension.

Responses to steady-state hypoxia and hypercapnia. Table I lists hemoglobin content, minute ventilation, and arterial blood pH, P_{O_2}, and P_{CO_2} during inhalation of air and 12% oxygen by seven goats. Where a range is indicated for hemoglobin level, each point represents the average of from two–four studies. In five of the seven goats, ventilation while breathing room air was not affected by anemia. Goats III and VII increased their ventilation with progressive anemia. For the group as a whole, there were no significant changes in ventilation or the pH, P_{O_2}, and P_{CO_2} of arterial blood during room air breathing after the induction of anemia. Severe anemia caused tachycardia but no significant change in systemic arterial blood pressure (Table II).

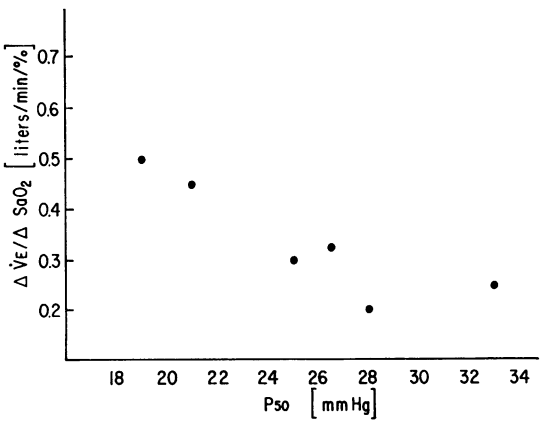

FIGURE 5 The response to transient hypoxia ($\Delta\dot{V}_E/\Delta SaO_2$ as defined in Fig. 3) is plotted against the affinity of goats hemoglobin for O_2, expressed as P_{50} (see text). A decreased affinity (increased P_{50}) was associated with a decreased response.

At normal hemoglobin levels, all seven animals increased their ventilation at the end of 6 min of inhalation of 12% oxygen. This increase averaged 1.76 liter/min. Essentially the same response was noted in the presence of moderate anemia (hemoglobin 7–8 g/100 ml of blood). The average increase in ventilation caused by breathing 12% oxygen was 1.62 liter/min.

When severe anemia was produced (hemoglobin 2.5–4.5 g/100 ml of blood), two patterns of response were seen. These are shown in Fig. 7. The first pattern, exhibited by four goats, consisted of an increase in ventilation after 1.5 min of inhalation of 12% oxygen which persisted at the end of 6 min. The increases in ventilation were not significantly different from values observed at normal hemoglobin levels. In three of the seven goats there was an increase in ventilation at the end of 1.5 min but ventilation dropped below base-line levels after 6 min. There was no apparent change in the animals' consciousness or in systemic arterial blood pressure (Table II) at the time this ventilatory depression was observed. However, in each of these three studies systemic oxygen consumption and CO_2 production were observed to fall at 6 min of inhalation of 12% oxygen, resulting in a fall in arterial P_{CO_2} in two of these animals and an unchanged arterial P_{CO_2} despite a fall in minute ventilation in the third.

The ventilatory responses to CO_2 inhalation were unaffected by anemia in all animals (Table III).

TABLE I

Ventilatory Response to 12% Oxygen

Goat no.	Hb	Air						12% O_2					
		$\dot{V}_E$	P_{O_2}	pH	P_{CO_2}	$\dot{V}_{O_2}$	RQ	$\dot{V}_E$	P_{O_2}	pH	P_{CO_2}	$\dot{V}_{O_2}$	RQ
	g/100 ml	*liter/min*	*mm Hg*		*mm Hg*	*cm³/min*		*liter/min*	*mm Hg*		*mm Hg*	*cm³/min*	
I	9.5–10.1	6.65	87.9	7.47	33.1	222	0.82	8.71	37.5	7.49	31.2	361	0.79
	7.2– 8.0	7.10	77.4	7.48	36.3			8.19	38.0	7.52	33.0		
	4.0– 4.5	7.11	80.5	7.43	37.4	310	0.80	7.13	41.5	7.49	30.1	242	0.77
II	9.0–10.2	5.45	91.6	7.44	41.0	186	0.75	7.02	41.1	7.46	37.4	193	0.80
	8.1	6.15	87.2	7.40	42.5	226	0.97	7.25	35.6	7.48	38.0	289	0.89
	4.6– 4.8	4.30	93.9	7.45	38.1	198	0.87	6.43	37.1	7.51	34.6	249	0.89
III	9.3	3.16	73.0	7.48	38.0	132	0.75	5.10	39.3	7.56	35.2	223	0.74
	7.5	4.73	86.4	7.56	35.2	169	0.75	9.65	36.6	7.58	31.1	330	0.81
	2.6– 3.1	5.30	96.0	7.48	35.0	187	0.90	4.37	37.2	7.51	31.2	103	1.00
IV	9.8	8.75	84.2	7.40	37.0	309	0.83	9.82	34.5	7.49	32.4	324	0.83
	6.5	5.59	80.8	7.42	36.8			7.73	40.7	7.51	32.7		
	4.5	5.80	74.9	7.44	37.7	181	0.73	6.82	36.2	7.48	36.5	197	0.77
V	9.0–10.0	5.71	68.1	7.40	41.9	254	0.84	8.40	34.0	7.47	38.5	295	0.86
	7.9– 8.2	9.01	76.2	7.45	41.0	211	0.73	8.84	38.0	7.48	38.3	278	0.69
	4.8	7.82	78.2	7.46	37.5	292	0.75	7.42	42.0	7.49	37.6	177	0.90
VI	9.0–12.8	7.71	65.0	7.45	38.6	182	0.75	8.30	30.1	7.49	36.4	260	0.71
	6.9	7.59	64.0	7.39	43.0			8.98	33.0	7.45	38.0		
	2.8– 3.8	7.96	68.0	7.46	34.0	160	0.74	8.26	37.0	7.51	30.9	182	0.71
VII	9.5–10.1	2.90	79.2	7.42	38.6			4.41	44.7	7.42	34.7		
	7.5	3.75	71.5	7.44	35.9			4.46	40.0	7.47	35.3		
	3.6– 4.8	7.27	75.2	7.43	33.3	190	0.65	8.63	40.5	7.49	29.3	200	0.74

DISCUSSION

This study was done to assess the ways in which the ventilatory response to hypoxia may be altered by anemia. Although this question has been explored in the past (*2*, 3), a re-examination seemed worthwhile for two reasons. First, only one study has examined severe anemia (hemoglobin concentrations of less than 5 g/100 ml of blood); and secondly, we believed that responses to rapid transient hypoxia would provide a closer estimate of peripheral chemoreceptor responses in unanesthetized animals than would responses to steady-state hypoxia, since the latter must include the central nervous system-mediated depression of ventilation which might be enhanced in the presence of severe anemia.

Effect of anemia on the ventilatory response to transient hypoxia. The basic finding of these studies was a uniform increase in responsiveness to transient hypoxia as a result of anemia. One complicating feature was the

TABLE II

*Circulatory Responses to 12% Oxygen**

Goat no.	Normal (Hb 10.0–12.0 g/100 ml)				Severe anemia (Hb 2.6–5.0 g/100 ml)			
	Air		12% O_2		Air		12% O_2	
	HR‡	BP	HR	BP	HR	BP	HR	BP
	min⁻¹	*mm Hg*	*min⁻¹*	*mm Hg*	*min⁻¹*	*mm Hg*	*min⁻¹*	*mm Hg*
II	81	94/70	103	98/74	91	92/71	146	106/76
III	84	120/71	108	120/71	162	122/82	180	123/75
IV	116	117/85	170	123/92	128	100/80	160	103/82
V	114	94/70	148	98/74	132	92/71	160	106/76
VI	93	125/76	132	132/81	117	105/50	139	105/50

* Each value represents the average of 2–3 determinations.

‡ HR, Heart Rate; BP, systolic/diastolic blood pressure.

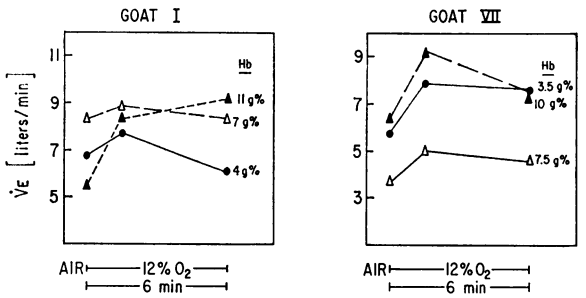

FIGURE 7 Two different effects of anemia upon the ventilatory response ($\dot{V}_E$) to inhalation of 12% O_2 are illustrated. In one (goat VII) inhalation of 12% O_2 resulted in a sustained (for 6 min) increase of ventilation at all levels of hemoglobin. In the other (goat I), the increase in ventilation which occurred after 1.5 min of breathing 12% O_2 was not sustained after 6 min when the animal was anemic. g% = g/100 ml.

fall in arterial P_{CO_2} which accompanied the hyperventilation. However, it is unlikely that the change in arterial P_{CO_2} influenced the results since the ventilatory responses were too rapid for the concomitant fall in P_{CO_2} to significantly alter ventilatory drive. In addition, since greater ventilatory responses would be associated with greater falls in P_{CO_2}, failure to control P_{CO_2} would have tended to obscure rather than produce the observed differences. Thus, we have interpreted the enhanced responses to transient hypoxia during anemia as indicating an enhanced responsiveness of the peripheral arterial chemoreceptors to this stimulus.

TABLE III
Ventilatory Response to CO_2

Goat no.	Hb	$\Delta\dot{V}_E/\Delta P_{CO_2}$
	g/100 ml	*liters/min/mm Hg*
I	10.5	2.93
	7.0	3.07
	4.0	2.28
II	10.2	1.84
	6.6	2.11
	4.8	2.28
III	10.0	1.38
	7.3	1.42
	2.6	1.93
IV	9.8	0.87
	6.5	0.83
	4.5	0.95
V	11.8	2.51
	7.0	2.60
	4.0	2.64
VII	10.0	4.41
	4.2	3.30

Paintal (16) and Mills and Edwards (17) have found increased neural discharges from arterial chemoreceptors after the lowering of blood oxygen content by the induction of carboxyhemoglobinemia. Since a similar decrease in oxygen content also occurs in anemia, this mechanism warrants consideration as a possible explanation for our findings. That this conclusion is not tenable seems evident for several reasons. First, other investigators have failed to demonstrate that carboxyhemoglobinemia increases chemoreceptor discharge (18). Secondly, this explanation does not account for the observation that euoxic ventilation was unaffected by severe anemia in five of seven animals. Thirdly, if the oxygen content of arterial blood were the primary chemoreceptor stimulus in the transient hypoxia studies, the relationship between change in ventilation and change in oxygen content should have approximated a single function. It is readily apparent, however, that the lines representing different hemoglobin levels in Fig. 2 would be even more divergent if arterial blood oxygen content rather than saturation were used as the ordinate. Finally, the observation that the ventilatory responses to transient hypoxia varied with O_2 affinity of hemoglobin when the responses were measured as the increase in ventilation per unit fall in O_2 saturation, but were a single function when ventilation was plotted against O_2 tension (Fig. 6), suggests that the peripheral sensory mechanism is sensitive to O_2 tension rather than to O_2 availability in arterial blood.

An increase in sympathetic nervous system activity produced by anemia could have contributed to the heightened chemoreceptor responses to hypoxia. Several features of our studies are in accord with this view. Thus, the data in Table II indicate that the animals manifested the expected tachycardia when anemic. This tachycardia is mediated mainly by an increase in the tone of the sympathetic nervous system (19). It has also been shown that catecholamine infusion enhances the ventilatory response to hypoxia (20). The reversal of the enhanced response to transient hypoxia during anemia by alpha-adrenergic blockade (Fig. 4) strongly supports this hypothesis. The failure of phenoxybenzamine to modify the ventilatory response to hypoxia in nonanemic animals suggest that the alpha-adrenergic system plays no role in the peripheral chemoreceptor-mediated response to hypoxia when hemoglobin levels are normal.

Steady-state hypoxia. There was considerable variability in responses of anemic animals to breathing 12% O_2 for 6 min. However, in contrast to the regular increase in responses to transient hypoxia, anemia never caused an increase of responses to steady-state hypoxia in the same animals. This statement may be made

whether one uses changes in minute ventilation or changes in arterial blood P_{CO_2} (in effect, changes in the ratio of metabolic rate to alveolar ventilation) as the index of ventilatory response to steady-state hypoxia. If changes in minute ventilation are considered as the appropriate index of response, one would conclude that anemic animals had either an unchanged or diminished response to steady-state hypoxia in comparison to the nonanemic state. If changes in arterial P_{CO_2} are considered as the appropriate index of response, one would conclude that the responses to steady-state hypoxia were unchanged by anemia. This discrepancy results from the fact that in two of the three anemic animals in which there was no increase in ventilation after 6 min of breathing 12% O_2 in N_2, there was a fall in arterial P_{CO_2} as a result of a fall in metabolic rate.

We postulate that the absence of an increased ventilatory response to steady-state hypoxia in anemic animals may be explained by the enhancement, during severe anemia, of the central nervous system-mediated ventilatory depressant effect of hypoxia. In all animals this phenomenon appears to have been sufficient to have, at least, counterbalanced the increased sensitivity of the peripheral chemoreceptors to hypoxia. In view of the many factors which influence oxygen delivery to the brain, it is not surprising that there was considerable variability in the ventilatory response when steady-state hypoxia was superimposed upon severe anemia. It is also not totally unexpected that metabolic rate fell during combined severe anemia and hypoxia in three of the seven animals since the O_2 delivery to tissues was likely to have fallen well below the levels described as "critical" for maintenance of O_2 consumption in isolated organ preparations (21, 22). The inconstancy of this finding is also probably related to the many determinants of O_2 availability at the tissue level which were not measured in this study.

The nature of the central nervous system-mediated depression of ventilation by hypoxia is speculative. Our previous studies have indicated that the phenomenon may be related to impairment of the oxidative metabolism of the brain (23), which, in turn, is related to the availability of oxygen to this organ. This would suggest that the oxygen delivery to the brain was adequate to maintain full tissue respiration when a nonanemic animal was made hypoxic but was inadequate when hypoxia was induced in the presence of severe anemia. Such a hypothesis remains to be tested directly. However, it may be of interest that in two studies, it has been shown that brain oxidative metabolism is lower than normal in the presence of severe chronic anemia (24, 25). It should be noted that other authors have ascribed the phenomenon to complex interactions at higher (i.e., suprapontine) brain centers rather than to a metabolic phenomenon (26).

Previous studies. The present data is reconcilable with those of previous studies. Bartlett and Tenny (2), using steady-state methods, found no effect of anemia upon the ventilatory response to hypoxia. However, they produced a much lesser degree of anemia in their animals (less than 50% reduction in packed erythrocyte volume) than in the present study. We found significant ventilatory depression with steady-state hypoxia or increase in responses to transient hypoxia, mostly when hemoglobin levels were reduced by more than 50%.

Cropp (3) found enhancement of the ventilatory response to steady-state hypoxia in a study of lightly anesthetized dogs. Although he produced a degree of anemia comparable to ours, there may be an important species difference. Dogs are able to raise erythrocyte levels of 2,3-diphosphoglycerate in the presence of tissue hypoxia (12). This enables them to rapidly decrease the oxygen affinity of hemoglobin and augment oxygen delivery to the tissues. The time which elapsed between the bleeding of the dogs and the testing of the response to hypoxia in Cropp's study was sufficient for this to take place. Goats, on the other hand, do not have this mechanism, and although they eventually are capable of producing equivalent rightward shifts of oxyhemoglobin dissociation curves, they do this by the time-consuming process (several weeks) of synthesizing a new species of hemoglobin with a lesser affinity for oxygen (13). Thus, a possible explanation for the difference between Cropp's findings in dogs and the data of the present study of goats may be as follows: peripheral chemoreceptor responses to combined anemia and hypoxia are the same in both species, since, as the present data indicate, these receptors seem to respond to O_2 tension of arterial blood rather than to O_2 delivery or availability. However, the effects of combined anemia and hypoxia upon the brain were greater in the goat because of its inability to rapidly augment O_2 delivery to tissues. Therefore, in goats, the central nervous system-mediated depressant effects of hypoxia may have been more severe and more likely to obscure the peripheral chemoreceptor-mediated stimulation of ventilation during steady-state studies than in dogs.

Similar considerations complicate the clinical application of this study. If severely anemic humans respond to hypoxia in a manner similar to our unanesthetized goats, then our data suggest that such subjects are at great risk from hypoxia and should be promptly treated with oxygen whenever pulmonary pathology impairs gas exchange. If, on the other hand, humans respond in a manner similar to anesthetized dogs, the

risk is probably not as great. It may be of interest that we have observed a severely anemic patient with chronic renal insufficiency (hemoglobin 3.8 g/100 ml of blood) who hypoventilated when he became hypoxic due to interstitial pulmonary edema. This hypoventilation was promptly reversed by administration of oxygen.

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