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EFFECT OF OXYGEN DESATURATION FROM CARBON MONOXIDE INHALATION ON ERYTHROCYTIC 2,3-DPG[1]

J. M. RAMSEY and BONNIE Z. KORCHMAROS, Biology Department, University of Dayton, Dayton, OH 45469

Abstract. Erythrocytic 2,3-diphosphoglycerate (2,3-DPG), in response to carbon monoxide (CO) hypoxemia, was studied in 12 rabbits exposed to 250 ppm CO for 3 hours. Each animal served as its own control. Individual determinations of carboxyhemoglobin, arterial oxygen content, oxygen saturation, and 2,3-DPG were made before and immediately after exposures to both CO and normal air. After CO inhalation, there was no significant change in 2,3-DPG despite an average reduction of 22% in arterial oxygen saturation. Moreover, there was no significant correlation between individual oxygen desaturations and changes in 2,3-DPG. The data imply that the 2,3-DPG mechanism offers little or no compensation for this form of hypoxia.

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Several causes of hypoxia, such as altitude exposure (Torrance *et al* 1970), ventilatory impairment (Edwards and Cannon 1972) and anemias (Eaton and Brewer 1968), are associated with increases of 2,3-diphosphoglycerate (2,3-DPG) in red blood cells. Since 2,3-DPG decreases the affinity of hemoglobin (Hb) for oxygen (Chanutin and Curnish 1967), an increase would shift the oxygen-dissociation curve to the right, promoting tissue oxygenation and thereby offering compensation for the hypoxia.

Tobacco smoking and automobiles in modern society have increased the inhalation of carbon monoxide (CO), a common source of induced hypoxia. Studies of the effect of CO exposures on 2,3-DPG have produced varying results. Dinman *et al* (1970) exposed rats for only 10–15 minutes to high levels of CO. About half the animals showed some increase in 2,3-DPG, whereas the others did not. Astrup (1970) found that 4 of 8 human volunteers reduced their 2,3-DPG after a brief CO exposure but the study had no controls. Morena *et al* (1974) exposed 10 rabbits for 10 minutes to CO (6–13% COHb) and observed a slight mean decrease in 2,3-DPG. Ramsey and Casper (1976) found no mean change in 2,3-DPG in 12 rabbits at any 3 hour interval during 24 hours following an exposure producing 20% COHb.

In most of the above mentioned studies the exposures were markedly brief, and no assessment was made of the actual O_2 deficit. Benesch *et al* (1968) showed that deoxyhemoglobin binds free 2,3-DPG, thereby stimulating erythrocytic glycolysis to synthesize more. This may explain why subjects at altitude produce more 2,3-DPG. Our study was designed to probe three questions: 1) Do a few hours of CO exposure resulting in 20–25% COHb produce a significant change in 2,3-DPG? 2) How much arterial O_2 desaturation results from such exposures? 3) Is there a significant statistical relationship between O_2 desaturation and changes in 2,3-DPG?

METHODS AND MATERIALS

Twelve New Zealand rabbits, weighing 4–5 kg, were selected for the study. They were exposed, one at a time, to normal air for 3 hours in a specially constructed exposure chamber. Blood was withdrawn from the ear artery and the ear vein with needle and syringe before, and immediately after, the 3 hour period. Determinations for Hb content (g/100 ml), COHb (%) and 2,3-DPG (μmole/ml) were done for each venous sample and O_2 content (vol %) was measured from arterial samples. Some of each arterial sample was perfused with 100% O_2 in order to measure the full O_2 capacity (vol %). The original O_2 content value then was divided

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by the capacity value in order to obtain the % saturation. The theoretical % saturation was calculated by multiplying the grams of Hb by 1.36 ml, the amount of O_2 which binds to a gram of rabbit Hb. The average Hb content in rabbits is only about 12.5 g/100 ml. Consequently, rabbit blood has less O_2 capacity than normal human blood. In the rabbit, a high cardiac output tends to compensate for this as well as rabbit oxyhemoglobin which dissociates at a higher O_2 tension (P_{50}=31.6).

Hb content was measured by the cyanmethemoglobin method and read spectrophotometrically. COHb was determined colorimetrically by the method of Trinder and Harper (1962), and O_2 content was measured in a motorized Natelson microgasometer, model 650. The quantification of 2,3-DPG was done by the method of Rose and Liebowitz (1970).

In another series of experiments each rabbit was subjected to the same routine again. This time the exposure chamber was perfused with 250 ppm CO, which in 3 hours should produce about 20–25% COHb.

RESULTS

Mean values for COHb, O_2 content, O_2 saturation, and 2,3-DPG for both the control series and the CO exposures are shown in table 1. No significant mean changes were found in any parameter when the animals breathed air only. Even though the CO exposure increased the average COHb to 25% and resulted in a mean O_2 desaturation of 22%, 2,3-DPG content showed no significant change. Furthermore, when individual 2,3-DPG changes are regressed on individual O_2 desaturations, no significant correlation was found (fig. 1).

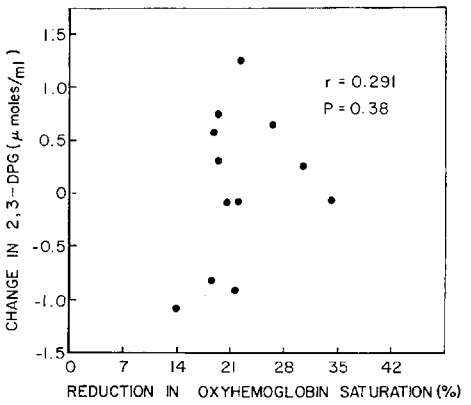

FIGURE 1. Scattergram illustrating the relationship of 2,3-DPG changes to oxygen desaturation in 12 rabbits exposed to 250 ppm carbon monoxide for 3 hours.

DISCUSSION

The results of this experiment confirm the conclusion of Ramsey and Casper (1976) that 2,3-DPG neither compensates nor aggravates the hypoxemia induced by CO inhalation. Even though a significant degree of O_2 desaturation occurs in this form of hypoxia, the Hb that is left without O_2 is not reduced. It is bound to CO. Apparently, this occupation precludes the binding of free 2,3-DPG to Hb, which in turn restricts the synthesis of new 2,3-DPG, thereby limiting the level of free 2,3-DPG in red cells. In addition to the reciprocal binding of O_2 and 2,3-

TABLE 1

Carboxyhemoglobin, arterial oxygen content, oxygen saturation, and 2,3-DPG in 12 rabbits exposed to 250 ppm CO for 3 hours.

	Controls		Exposed	
	Before	After	Before	After
Carboxyhemoglobin (%)	1.21±0.31*	1.23±0.39	1.24±0.30	25.3 ±5.48**
Oxygen Content (vol %)	15.9 ±1.01	16.1 ±0.98	16.0 ±1.04	12.3 ±1.82**
Oxygen Saturation (%) (measured)	93.5 ±3.42	93.9 ±3.36 (7 increased, 5 decreased)	93.7 ±3.43	72.1 ±4.27** (12 decreased)
Oxygen Saturation (%) (calculated)	95.2 ±2.31	95.6 ±2.02	95.4 ±2.35	72.8 ±2.95**
2,3-DPG (μmoles/ml)	2.23±0.70	2.26±0.63 (6 increased, 6 decreased)	2.27±0.67	2.33±0.98 (5 increased, 7 decreased)

*Mean±standard deviation.
**Significant $P \leq 0.001$.

DPG by Hb, it appears likely that CO is competitive with 2,3-DPG as well as with O_2. In order to confirm this, controlled *in vitro* studies of these three substances are in order.

Despite the absence of intraerythrocytic adaptation to the tissue hypoxia induced by carbon monoxide, there are other operant compensations. Ramsey (1975), Sagone and Balcerzak (1975), and Smith and Landow (1978) all showed that carbon monoxide hypoxemia promotes an increase in the red cell mass. This adaptation, however, develops only with chronic exposures not acute ones. In general, there is not a great deal of adaptive tolerance to carbon monoxide hypoxemia, especially when compared to other types of oxygen reduction, such as that resulting from exposure to hypobaric atmospheres.

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