Effect of Hypoxia and Hypercapnia Alone and in Combination upon the Circulating Red Cell Volume of Rats[1] (35407)

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Exposure to hypoxia results in increased hematocrit and red cell counts. Recently, increases in erythrocyte stimulating factor (ESF) have also been reported during hypoxia (1–3). The hypoxia-stimulated increase in Fe uptake, an indicator of erythropoiesis, however, was almost completely blocked by addition of 5% CO_2 to the hypoxic gas mixture (4). The degree of reticulocytosis was also lower during exposure to 10% O_2 + 5% CO_2 than with 10% O_2 alone (5). A smaller rise in hematocrit and histological evidence of depressed levels of erythropoiesis occurred in rats inspiring 70 Torr P_{O_2} + 60 Torr P_{CO_2} compared with rats inspiring this level of oxygen with no CO_2 (6).

Although hypercapnia apparently does influence erythropoiesis there is little information as to the degree of this effect upon the number of circulating red cells either in the normoxic or hypoxic state. The present experiment was conducted to measure the circulating red cell volume (CRCV) in rats exposed up to 24 days to either hypoxia (70 Torr $P_{I_{O_2}}$), hypercapnia (60 Torr $P_{I_{CO_2}}$), or a combination of this level of hypoxia and hypercapnia.

Methods. Young adult male Charles River CD* strain rats were used as experimental animals. Housing and care of the animals and a description of the environmental chamber have been published previously (6). The ambient temperature was maintained at 24–25°, relative humidity 40–50%, total barometric pressure 380 Torr, O_2 fraction at .21 or .42 and CO_2 fraction at 0 or .18. The three experimental environments are shown in Table I. Fifty animals were placed in the chamber for each run. They were removed in groups of 10 after 1, 3, 8, 16, or 24 days' exposure for blood volume measurements. Twelve controls each were measured at 0 and 24 days. These rats were housed and cared for similarly to experimental animals except that they breathed room air at ground level.

Circulating red cell volume (CRCV) was measured using ^{51}Cr labeled red cells. The cells were labeled by addition of 7.43 μCi of Sodium-Chromate-^{51}Cr and of ACD solution to an amount of packed red cells obtained from 10 ml of whole blood. They were incubated at room temperature for 20 min. Upon completion of incubation the cells were washed three times then resuspended in saline restoring the original volume to 10 ml. One-half ml of this suspension was injected into the tail vein of each conscious rat immediately after removal from the experimental environment. Thirty min later the rats were anesthetized with ether, an abdominal incision was made and about 8 ml of blood were collected from the abdominal aorta. Two capillary tubes were filled at this time for a hematocrit determination. Five ml of the blood collected were measured in a gamma well counter (Nuclear Chicago). A standard was made by addition of 4.5 ml of saline to 0.5 ml of tagged blood. Total blood volume was calculated from the following equation:

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$$\text{Blood volume (BV) (ml)} = \left(\frac{\text{cpm of standard} \times 5}{\text{cpm of a 5-ml aliquot}}\right) - 0.5 \text{ ml.}$$

The 0.5-ml volume was subtracted to account for the amount of tagged blood injected. Percentage packed cell volume was obtained by multiplying the hematocrit by 0.96. This accounts for the trapped plasma. CRCV was then equal to BV $\times$ packed cell volume, and plasma volume was obtained by subtracting CRCV from BV. In calculating plasma volume, no correction factor was applied for uneven distribution of cells between central and peripheral blood. At present there is disagreement as to the size of this factor in the rat or whether it is even significant (7, 8).

Statistical analysis. Linear trends were fitted for each treatment. They were then tested to determine if the slopes were significantly different from zero, if they differed among treatment groups, and if they adequately fit a linear trend. The treatment groups were also compared at each time. Since there were detectable differences at each time, with the exception of CRCV on day 1, a Duncan's multiple range test (9) was performed on each of the variables for each time of exposure. Pair differences were determined at the 5 and 1% level of significance. The data presented represent 12 animals/group from each of 2 control groups and 10 animals/group in the experimental environments with the following exceptions: 9 animals/group, hypercapnia day 24, hypoxia–hypercapnia day 8; and 8 animals/group, hypoxia–hypercapnia days 1 and 16.

Results. Circulating red cell volume (CRCV) (Table II, Fig. 1). Control values at 0 and 24 days did not differ significantly, so they were combined to give a mean CRCV of 2.46 $\pm$ 0.30 ml/100 g wt. Linear trends differed among groups ($p<.001$). The hypoxic rats showed a highly significant increase in CRCV with time ($p<.001$), while a decrease with time occurred in the hypercapnic rats ($p<.001$). The combined treatment did not result in a significant trend but there were time differences which were shown as a significant deviation from linear ($p<.05$). CRCV of the hypoxic rats showed a significant increase above control levels after 1 day of exposure, but this increase was only about one-half that of the hypoxic–hypercapnic rats. The CRCV of the hypoxic rats, however, continued to increase throughout the run while the hypoxic–hypercapnic rats showed little change after the first day. As a result the hypoxic rats had a greater CRCV than all other groups from day 3 on. Hypercapnic rats showed an increase in CRCV after 1 day of exposure, then declined to control levels after 3, 8, 16 days and fell to less than controls after 24 days.

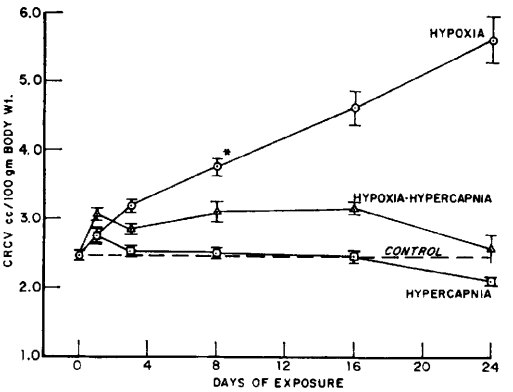

FIG. 1. Circulating red cell volume of rats exposed up to 24 days to hypoxia 70 Torr P_{O_2}, hypercapnia 60 Torr P_{CO_2} or hypoxia–hypercapnia, 70 Torr P_{O_2} 60 Torr P_{CO_2}.

TABLE I. Composition of the Experimental Environment.[a]

Run	% O_2	Environmental P_{O_2} Torr	Calculated inspired P_{O_2} Torr	% CO_2	Environmental P_{CO_2} Torr	Calculated inspired P_{CO_2} Torr
1	21	80	70	0	0	0
2	21	80	70	18	68	60
3	42	160	140	18	68	60

[a] Total barometric pressure, 380 Torr; temp, 24°; relative humidity, 40–50%.

TABLE II. Comparisons of Differences Between Means with p Values During Exposure of 1–24 Days to the Experimental Environments.

Duncan's Multiple Range Test (9).

Variable	Exposure (days)	Control; hyper-capnia	Control; hypoxia	Control; hypoxia–hypercapnia	Hyper-capnia; hypoxia	Hypercapnia; hypoxia–hypercapnia	Hypoxia; hypoxia–hypercapnia
CRCV/wt	1	$p<.05$	$p<.05$	$p<.01$	NS	$p<.05$	$p<.05$
	3	NS	$p<.01$	$p<.01$	$p<.01$	$p<.05$	$p<.01$
	8	NS	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$
	16	NS	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$
	24	$p<.01$[a]	$p<.01$	NS	$p<.01$	$p<.05$	$p<.01$
Packed cell vol %	1	NS	$p<.01$	$p<.01$	NS	$p<.01$	$p<.01$
	3	NS	$p<.01$	$p<.01$	$p<.01$	$p<.01$	NS
	8	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$
	16	$p<.05$	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$
	24	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$	$p<.01$
Plasma vol/wt	1	NS	NS	$p<.05$	$p<.05$	$p<.01$	NS
	3	NS	NS	$p<.01$	NS	$p<.01$	$p<.01$
	8	$p<.01$	$p<.01$	NS	$p<.01$	$p<.01$	$p<.01$
	16	$p<.01$	NS	NS	$p<.01$	$p<.01$	NS
	24	$p<.01$	$p<.01$	NS	$p<.01$	$p<.05$	NS

[a] Due to differences in variability it is possible for some pairs to differ significantly at days 16 and 24 even though their means differed less than other pairings which are not significantly different.

Percentage packed cell volume (Table II, Fig. 2). The packed cell volume of controls did not differ at 0 or 24 days, so they were combined to give a mean of 40.9 ± 2.7. All three treatments resulted in significant linear trends ($p<.001$) which differed from each other ($p<.001$); however, only the combined condition gave an adequate fit. Hypoxia resulted in steadily increasing red cell percentages throughout the run. The combined condition resulted initially in significantly increased red cell percentages, but, as with CRCV, all the increase occurred within 1 day, and from day 1 on the packed cell volume in this group showed a significant downtrend. Packed cell volume of the hypercapnic rats showed a significant downtrend with time, and pair testing indicated a decrease below control levels from day 8 on. This was primarily the result of an increase in plasma volume rather than a decrease in CRCV.

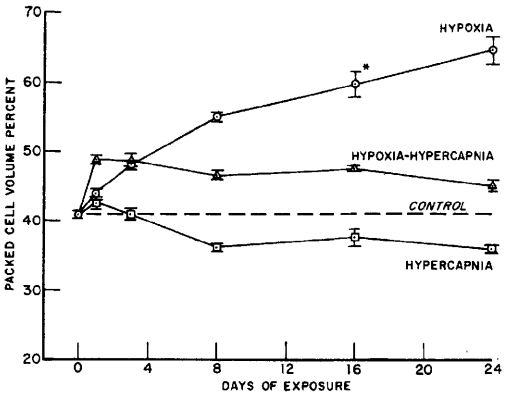

FIG. 2. Packed cell volume of rats exposed up to 24 days of hypoxia 70 Torr P_{O_2}, hypercapnia 60 Torr P_{CO_2} or hypoxia–hypercapnia 70 Torr P_{O_2} 60 Torr P_{CO_2}.

Plasma volume (Table II, Fig. 3). Plasma volume for control animals did not differ significantly at 0 or 24 days, so the data were combined to give an overall average of 3.54 ± 0.23 ml/100 g of body wt. Only the plasma volume of hypoxic rats adequately fits a linear trend. This downward trend was highly significant ($p<.01$). Although the other groups did not show a significant linear trend, their means did change significantly with time. After 1 and 3 days of exposure only the hypoxic–hypercapnic rats differed from controls showing a significant decrease in plasma volume. From days 8 through 24,

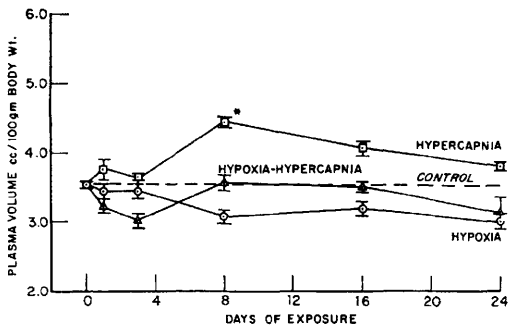

FIG. 3. Plasma volume of rats exposed up to 24 days of hypoxia 70 Torr P_{O_2}, hypercapnia 60 Torr P_{CO_2} or hypoxia–hypercapnia 70 Torr P_{O_2} 60 Torr P_{CO_2}.

hypercapnia resulted in a significantly increased plasma volume. During the same time period, plasma volume was significantly less than controls in the hypoxic rats, while exposure to both variables simultaneously did not result in a detectable difference from control levels.

Discussion. One possible reason for a failure of the CRCV to show continued increase beyond day 1 of hypoxia–hypercapnia is an alleviation of tissue hypoxia by the concomitant hypercapnia. Several factors could be involved, the most likely being an increase in alveolar P_{O_2} levels resulting from the additional respiratory stimulus of added CO_2. During acute hypoxia, addition of CO_2 did raise alveolar P_{O_2} levels even though the ambient oxygen percentage was diluted by this addition (10). Moreover, in a study recently completed but as yet unpublished, we have found that arterial P_{O_2} levels increased from a mean of 34 Torr in rats chronically inspiring 70 Torr P_{O_2} to 51 Torr in rats inspiring the same level of oxygen plus 60 Torr CO_2

Hypercapnia may also affect the position of the oxyhemoglobin dissociation curve. A sudden increase in CO_2 levels in the blood and resultant acidosis will shift the dissociation curve to the right, the well-known Bohr effect (11). In the chronic hypercapnic state, however, pH will partially recover (12). Moreover, the increased acidosis also results in a drop in 2,3-diphosphoglycerate (2, 3-DPG) (13) which in turn causes a leftward shift in the dissociation curve (14, 15). Recent work indicates that in the chronic hypercapnic state the partial pressure of oxygen at half saturation (P_{50}) is, in fact, near normal (16). During chronic hypoxia, on the other hand, blood levels of 2,3-DPG increase along with an increase in P_{50} (17). Whether a decrease in the affinity for oxygen, which aids in supporting tissue P_{O_2}, is actually the case in the chronic hypoxic–hypercapnic animal is yet open to question.

Another factor which could affect tissue oxygenation is metabolic rate. Any decrease would lessen oxygen usage and tend to increase tissue P_{O_2} levels. In the acute hypercapnic state oxygen uptake was found to be inversely related to arterial P_{CO_2} levels in both dogs and humans (18, 19). Again, there is no information concerning the chronic hypercapnic state.

The only definite evidence thus far that concomitant CO_2 aids tissue oxygenation is the increase in arterial P_{O_2} levels mentioned earlier. However, an arterial P_{O_2} of 51 Torr is still definitely in the hypoxic range. In an earlier study, immature rats exposed to the present hypoxic–hypercapnic conditions appeared extremely stressed, losing weight for a long period, then gaining much more slowly than the hypoxic or hypercapnic groups (6). If tissue hypoxia was not present in these hypoxic–hypercapnic rats, it seems unlikely that they would appear much more stressed than the rats that were exposed to hypercapnia alone.

The early increase in the CRCV of hypoxic rats has been attributed to a release of stored cells from the spleen and bone marrow into the circulation (20). Extreme hypercapnia also causes splenic contraction, an occurrence attributed to adrenal discharge (21). In the present experiment, hypoxia or hypercapnia caused about equal initial increases in CRCV. In the hypercapnic animals, the increase lasted only a short time with the extra red cells evidently being removed from the circulation. Hypoxia–hypercapnia, on the other hand, caused an early increase in CRCV equal to the sum of that caused by hypoxia or hypercapnia alone, and this level was nearly maintained throughout most of the experiment. The extremely small size of the spleen during hypoxia–hypercapnia (6) and a lack of increase in ^{59}Fe

uptake (2) indicate that the maintenance of this increase is due to continued splenic contraction rather than erythropoiesis. If there were no hypoxic stimulus, it is improbable that splenic contraction would continue, and these extra cells would have remained in the circulation for such a long period of time.

There are many ways that hypercapnia could inhibit erythropoiesis if such an inhibition actually occurs. Barlett and Phillips (5) suggested that since, under natural conditions, hypoxia is almost always accompanied by hypocapnia, perhaps low blood P_{CO_2} levels are a necessary part of the hypoxic stimulation of erythropoiesis. Faura *et al.* (2) found that hypoxic–hypercapnic mice do respond to erythropoietin, so that any inhibition is at the level of production or release of this substance. Prolonged weight loss during hypoxia–hypercapnia (6) suggested inanition which itself inhibits release of erythropoietin (22). The apparently high stress levels caused by hypoxic–hypercapnic conditions could also easily affect levels of testosterone, adrenal corticoids, thyroxine, and pituitary hormones, all of which have been shown to influence erythropoiesis (23).

Summary. Three groups of 50 rats each were exposed for periods of up to 24 days to either hypoxia (70 Torr $P_{I_{O_2}}$), hypercapnia (140 Torr $P_{I_{O_2}}$, 60 Torr $P_{I_{CO_2}}$), or a combination of this level of hypoxia and hypercapnia.

The combined condition resulted in a much smaller rise in circulating red cell volume per body weight than in rats exposed to hypoxia alone. The increase that did occur could be mainly attributed to a release of stored red cells into the circulation. During hypercapnia, plasma volume increased with only small changes in numbers of circulating red cells. This resulted in a depression of hematocrit.

It is still not clear whether concomitant hypercapnia increased oxygen levels in the hypoxic–hypercapnic rats sufficiently to remove the erythropoietic stimulus, or if CO_2 actually inhibited erythropoiesis.

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