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SUPERIMPOSITION OF CARBON DIOXIDE ON ACUTE ISOBARIC HYPOXIA:
PLASMA ERYTHROPOIETIN, ACID-BASE STATUS, AND P_{50}
IN THE UNANESTHETIZED RABBIT

Joachim Wolf-Priessnitz
(Ph.D thesis)

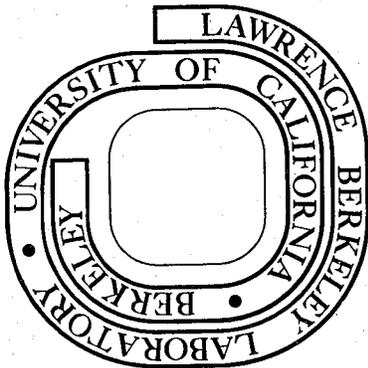
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IN THE UNANESTHETIZED RABBIT

by

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To Ms. Lynn Mahlmann for her advice, encouragement and technical assistance, given unselfishly during the entire duration of this project.

To Dr. Mary Barker for her important contribution of scientific editing and to Ms. Joan Graham for her excellent secretarial assistance.

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SUPERIMPOSITION OF CARBON DIOXIDE ON ACUTE ISOBARIC HYPOXIA:

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ABSTRACT

Major factors affecting changes of the arterial Hb- O_2 affinity (P_{50}) were examined in relation to the initiation of erythropoietin (ESF) production in unanesthetized New Zealand white male rabbits. They were exposed to an isobaric hypoxic environment (8.8% O_2) with and without CO_2 (5.6% or 10%). During 5 hrs exposure, samples of arterial blood were collected for measurements of: 1) plasma ESF titers; 2) whole-blood pH, P_{CO_2} , S_{O_2} , lactate, pyruvate, and P_{50} ; 3) intraerythrocytic 2,3-DPG, ATP and ADP. Exposure to 8.8% oxygen alone stimulated ESF production and caused a leftward shift in the in vivo P_{50} ; the addition of CO_2 significantly inhibited ESF production and blocked the shift in $P_{50(i.v.)}$. The data suggest that increased oxygenation of the whole-body tissues occurs with exposure to 8.8% $O_2 + CO_2$ as reflected by lower whole-blood "excess lactate" accumulation. In keeping with the prevailing theory, the suppression of ESF production is probably a result of this increased oxygenation.

The shifts in $P_{50(i.v.)}$ generally followed changes in pH; lesser influences were exerted by changes in BE, MCHC, and possibly ADP. Increased tissue oxygenation with the hypoxic-hypercapnic exposures is probably the result of increased pulmonary ventilation coupled with facilitated oxygen unloading to the tissues caused by a relatively right-shifted $P_{50(i.v.)}$.

INTRODUCTION

Erythropoietin: a regulator of erythropoiesis.

Mammals require oxygen to survive. Physiological changes occur when the oxygen supply to the tissues is inadequate. Whether these changes alleviate tissue hypoxia when an animal is exposed to hypoxic conditions depends on the severity and duration of the hypoxic stress.

One of the important ways in which humans and many animal species increase their capacity to deliver oxygen to the tissues during hypoxic stress, is to increase the number of circulating red cells. Increases in red cell concentration, in response to high altitude exposure, was observed by Bert as early as 1882. Confirmation of this observation by a number of investigators (Krantz and Jacobson, 1970) led to the general acceptance of the theory that low oxygen supply to the marrow serves as a direct trigger for erythropoiesis. However, direct marrow oxygen measurements by Grant and Root (1952) disproved this hypothesis. An alternative hypothesis, already advanced in 1906 by Carnot and Deflandre, that erythropoiesis is regulated indirectly through a humoral agent, was confirmed by the classical parabiotic experiments by Reissmann (1950).

This humoral agent, erythropoietin (ESF, erythropoiesis stimulating factor), has been under investigation for the past twenty-five years. That the kidney is the primary site of ESF production was originally demonstrated in vivo by Jacobson et al. (1959) and by the use of isolated kidney perfusion preparations by Kuratowska et al. (1961). Since that time numerous studies have corroborated those findings. The renal tissue(s) involved in ESF production have not yet been isolated. Nor has an undisputed

model for the biogenesis of ESF been developed to fit all the experimental findings. The existence of a limited extrarenal ESF production site, demonstrated by exposing nephrectomized animals to very severe hypoxia (Goldwasser et al., 1958a; Schooley and Mahlmann, 1972), has added to the complexities of isolating the cells which produce ESF.

The primary ESF target is thought to be a primitive unipotential stem cell committed to the erythroid line. The major elements which comprise the increased erythropoiesis, as a result of ESF stimulation, are cellular amplification, through induced cell divisions, early reticulocyte release from the marrow, and increased hemoglobin (Hb) synthesis within the developing erythroblasts (Adamson and Finch, 1975).

Although ESF is present in normal plasma and urine, it is not detectable, unless concentrated, with the bioassay methods still used out of necessity (more sensitive assay systems are being developed, e.g., immunoassay, in vitro tissue culture, and hemagglutination inhibition techniques). Under hypoxic conditions, however, plasma ESF titers are frequently measurably elevated within a few hours from the time hypoxia is initiated. After several days, this increase in ESF is followed by increases in the erythropoietic parameters which reflect the effects of ESF stimulation, e.g., red cell concentration, reticulocytosis, etc.

Decreased oxygen availability, probably to the ESF producing cells plays a dominant role in triggering ESF production. Some studies suggest that ESF levels are directly related to the severity of hypoxia. This relationship has been demonstrated with anemia (Naets, 1959; Hammond et al., 1962; Hammond and Keighly, 1962; Gordon et al., 1964) and hypoxic hypoxia (Erslev, 1957; Gurney et al., 1965; Siri et al., 1966;

Carmena et al., 1967). Reversal of anemic hypoxia through hyperoxia and plethora has been shown to reduce the plasma ESF levels and decrease erythropoiesis (Krantz and Jacobson, 1970). Some evidence suggests that ESF production may be a function of oxygen availability relative to the oxygen requirements of the ESF producing cells. A lowered metabolic rate, observed with hypophysectomy, starvation, and hypothyroidism, decreases erythropoiesis (Krantz and Graber, 1974). Increasing the metabolic rate with thyroid hormone or dinitrophenol increases erythropoiesis (Jacobson et al., 1959); the thyroid stimulus can be blocked with anti-erythropoietin (Peschle et al., 1971).

The inhibition of erythropoiesis by CO₂ during hypoxia.

Prolonged exposure (days-weeks) to environmental hypoxia stimulates erythropoiesis. This erythropoietic response to hypoxia is nearly abolished when CO₂ is added to the hypoxic gas mixture. Exposure to 10% O₂ with 5% CO₂ added almost completely blocked the increase in ⁵⁹Fe uptake by the red cells (an indicator of erythropoiesis) when compared to ⁵⁹Fe uptake observed with 10% O₂ alone (Faura et al., 1968). When rats were exposed to low ambient oxygen pressure (P_{O₂}, 70 torr) plus CO₂ (P_{CO₂}, 60 torr) their hematocrits (Hct) did not increase as much and histologically erythropoiesis was depressed when compared to rats exposed to hypoxia without CO₂ (Pepelko, 1970). In additional experiments with the same gas mixtures, Pepelko (1971) observed that rats exposed to hypoxia alone (up to 24 days) increased their circulating red cell volume (CRCV) but with the hypoxic-hypercapnic exposure this increase in CRCV was very significantly depressed.

A plausible explanation for this erythropoietic suppression would be that the hypoxia-induced production of ESF is suppressed when CO₂ is added to the hypoxic gas mixture. This hypothesis is supported by the recent work of Schooley and Mahlmann (1975). These investigators observed almost complete suppression of ESF production in rats breathing a 10% O₂ + 10% CO₂ gas mixture (4 hrs) when compared to controls breathing 10% O₂ alone. The physiological events modifying ESF production were not examined. Hence, the problem presented by Krantz and Graber (1974) that "hypercapnea reduced the erythropoietic response to hypoxia, but that the mechanism of this effect is not known" has only been partially resolved. The purpose of this study is to determine the physiological mechanisms underlying the difference in ESF response of animals exposed to a hypoxic versus a hypoxic-hypercapnic environment.

Changes in the affinity of the hemoglobin for oxygen as an indirect modulator of erythropoietin production.

As discussed above, tissue hypoxia is the primary stimulus for the production of ESF. By implication, processes or conditions which alleviate tissue hypoxia reduce the stimulus and cause a decrease in plasma ESF levels. If this hypothesis is applied to the observation that rats exposed to a hypoxic-hypercapnic environment have lower levels of serum ESF than those exposed to hypoxia alone, the added CO₂ most likely alleviates tissue hypoxia.

Some experimental evidence suggests that changes in the affinity of Hb for oxygen (Hb-O₂ affinity) can sufficiently alter oxygen delivery to the tissues to modify ESF production.

The Hb- O_2 affinity is determined by the position of the oxygen dissociation curve. This position is defined by the oxygen pressure (P_{O_2}) in torr necessary to 50% saturate the Hb with oxygen (P_{50}). The in vivo P_{50} (hereafter designated as $P_{50(i.v.)}$) incorporates all factors which influence the Hb- O_2 affinity and is an estimate of the Hb- O_2 affinity under which an animal functions. In contrast, the in vitro P_{50} (hereafter designated as $P_{50(7.4)}$) is an expression of the Hb- O_2 affinity under conditions of constant pH (7.4), P_{CO_2} (40 torr), and temperature ($37^\circ C$).

Some ions and molecules function as ligands which interact preferentially with deoxyhemoglobin. When the concentration of these ligands increase, the Hb- O_2 affinity decreases, resulting in a "right-shifted" P_{50} ; a higher P_{O_2} is required to 50% saturate the Hb with oxygen. The opposite process occurs when the concentration of these ligands is reduced.

The best known of these ligands is the H^+ ion (Bohr et al., 1904). Changes in blood pH, even in a normoxic environment, can apparently shift the $P_{50(i.v.)}$ sufficiently to alter ESF production. In the rat, respiratory alkalosis was induced by the administration of cobalt (Miller et al., 1974) or acute bleeding (Miller et al., 1976). The rapid increase in pH caused a decrease in the $P_{50(i.v.)}$, followed by detectable increases in circulating ESF levels. During short term high altitude exposure (hrs) a similar pattern was observed in the human. However, when the leftward $P_{50(i.v.)}$ shift was blocked by simultaneous administration of acetazolamide (a carbonic anhydrase inhibitor), ESF production was suppressed (Miller et al., 1973). Using acetazolamide, Schooley and Mahlmann (1975) also observed partial blocking of the ESF response in the rat exposed to acute simulated high

altitude. In all these investigations, the authors speculate that the increase in Hb- O_2 affinity sufficiently decreases oxygen unloading to the tissues to either cause or increase tissue hypoxia, which in turn triggers the initiation of ESF production. Blocking this increase in Hb- O_2 affinity, therefore, should increase oxygen unloading to the tissues, thus alleviating tissue hypoxia. Therefore, the stimulus for ESF production should be reduced.

The above hypothesis outlines a mechanism which may explain why ESF production is inhibited in rats exposed to a hypoxic-hypercapnic environment. When an animal is initially exposed to hypoxia alone, the decrease in the P_{O_2} of the blood triggers hyperventilation (Slonim and Hamilton, 1971). Hyperventilation causes a decrease in the blood P_{CO_2} which results in respiratory alkalosis (Davenport, 1971). In the absence of other compensatory mechanisms, respiratory alkalosis shifts the $P_{50(i.v.)}$ to the left (see above), thereby decreasing oxygen unloading to the tissues. Addition of CO_2 to the hypoxic gas mixture should decrease or prevent the respiratory alkalosis. Hence, the leftward $P_{50(i.v.)}$ shift observed with a brief hypoxic exposure (Astrup et al., 1970; Miller et al., 1973) would be partially or completely blocked when CO_2 is added to the hypoxic gas mixture. Possibly this relatively right-shifted $P_{50(i.v.)}$ enhances oxygen unloading to the tissues, thereby reducing the stimulus for ESF production.

Not all experimental observations are consistent with the view that the position of the $P_{50(i.v.)}$ can affect the production of ESF. The administration of cyanate (NaOCN) to rats caused a leftward shift in the $P_{50(i.v.)}$ but did not trigger an ESF response (Miller, 1975). Nor was ESF production suppressed in the rat by uremic acidosis (Schooley and Mahlmann, 1975) during exposure to simulated high altitude, even though the acidosis was probably severe enough to prevent the leftward $P_{50(i.v.)}$

shift normally observed with acute high-altitude exposure. Whether a relatively right-shifted $P_{50(i.v.)}$, anticipated when CO_2 is added to a hypoxic gas mixture, can have an impact on ESF production will be explored in the present study.

The effect of 2,3-diphosphoglycerate on the affinity of hemoglobin for oxygen.

It has been known since 1925 (Greenwald, 1925), that pig red cells contain very high levels of 2,3-diphosphoglycerate (2,3-DPG). Studies by Rapoport and Guest (1941) showed that such high 2,3-DPG levels were not unique to the pig, but were present in the human and several other animal species.

No functional significance was attached to these observations until the discovery by Benesch and Benesch (1967) that, in dilute Hb solutions, organic phosphates decrease the binding affinity of Hb for oxygen. The same effect is seen at the higher 2,3-DPG concentrations found in human erythrocytes (Chanutin and Curnish, 1967), where 2,3-DPG and Hb are present in approximately equimolar concentrations (Benesch and Benesch, 1969). Organic phosphates other than 2,3-DPG also decrease the Hb- O_2 affinity. However, their concentrations in the intact red cell are much lower, hence they usually have less impact on the P_{50} than 2,3-DPG.

The results of numerous studies have established that intraerythrocytic 2,3-DPG concentrations increase in the human and several animal species during exposure to real or simulated high-altitude. Furthermore, these 2,3-DPG increases appear to resolve the longstanding puzzle of why the $P_{50(7.4)}$ increases in high altitude residents (Keys *et al.*, 1936; Aste-Salazar

and Hurtado, 1944). Lenfant et al. (1968) showed that humans exposed to high-altitude increased their $P_{50(7.4)}$ within 12 hrs; this increase was paralleled by an increase in 2,3-DPG. Both the rat and guinea pig increased their $P_{50(7.4)}$ within 36 hrs during high altitude exposure. These increases were totally accounted for by the rise in 2,3-DPG, as verified under in vitro conditions (Baumann et al., 1971). In the rat, a more severe hypoxic environment stimulates 2,3-DPG production and causes an increase in the $P_{50(7.4)}$ within 5 hrs (Schooley and Mahlmann, 1975).

These 2,3-DPG increases are probably caused primarily by the alkalosis attendant with exposure to environmental hypoxia (Asakuta et al., 1966; Bellingham et al., 1971; Duhm and Gerlach, 1971). The opposite effect is observed when respiratory acidosis, caused by addition of CO_2 to normoxic gas mixtures, causes a decrease in 2,3-DPG in the guinea pig (Messier and Schaeffer, 1971) and rabbit (Rand et al., 1973). In the rat, the rise in 2,3-DPG observed with exposure to hypoxia, is completely abolished when 5% CO_2 is added to the hypoxic gas mixture (Duhm and Gerlach, 1971).

If the addition of CO_2 to a hypoxic gas mixture relieves tissue hypoxia by way of a relative increase in the $P_{50(i.v.)}$, it is likely that changes in 2,3-DPG either contribute nothing to, or possibly even counteract, this shift. One of the aims of the present study will be to determine what contribution is made by some of the intraerythrocytic organic phosphates towards shifting the $P_{50(i.v.)}$ during hypoxia-hypercapnia.

Experimental goals.

Experimental evidence suggests that blocking the respiratory alkalosis attendant with exposure to environmental hypoxia may cause an

increase in tissue oxygenation sufficient to suppress the increase in ESF production normally observed with hypoxia alone. Such ESF blockage has been observed in rats breathing a hypoxic gas mixture when CO_2 is added. It is the purpose of this study:

1) To determine if the initiation of ESF production is blocked in rabbits briefly exposed to a hypoxic-hypercapnic environment.

2) To ascertain if changes in plasma ESF levels reflect an all-or-none release of stored hormone in response to this hypoxic stimulus. Or, whether ESF increases are dependent on a continuous hypoxic stimulus.

3) To establish whether differences in ESF production, between rabbits exposed to hypoxia with or without CO_2 , could be attributed to differences in the degree of tissue hypoxia developed by the two groups.

4) To relate differences in tissue hypoxia and ESF levels to changes in the $\text{P}_{50(\text{i.v.})}$.

5) To determine the relative influence of changes in H^+ and intra-erythrocytic organic phosphates on the P_{50} .

MATERIALS AND METHODS

Animals.

Male New Zealand white rabbits (Oryctolagus cuniculus), weighing between 2 and 3 kg were housed for a minimum of 3 months in our laboratory animal facilities prior to use. Standard laboratory rabbit chow and water were provided ad libitum. Only healthy animals, indicated by normal weight gain and appearance, weighing between 3 and 4-1/2 kg at the time of experimentation, were used. No animal was used for more than 2 experiments; a minimum of 3 months was allowed for recovery between experiments.

Experimental protocol.

1) In order to test the suppressive effect of CO₂ on the ESF response to hypoxia under isobaric conditions, it was first necessary to determine an appropriate hypoxic stimulus. Exposure of mice and rats in a decompression chamber to a simulated altitude of 22,000 ft for extended periods of time is a standard procedure in this laboratory. This severe hypoxic stress is non-life threatening, and causes both a rapid and significant increase in plasma ESF levels in these animal species. Preliminary experiments were carried out to determine the response of the rabbit to these hypoxic conditions during a prolonged exposure period.

Pairs of unrestrained rabbits, provided with food and water, were exposed to 22,000 ft simulated altitude for 8, 12, 16, 20, 24, 36 or 48 hrs; 30 min were allowed for both decompression and recompression. Immediately following the hypoxic exposure, rabbits were bled from the auricular artery (10-15 ml), and the plasma stored at -20^o C for determination of plasma ESF levels.

2) On the basis of the results of the above experiments, a gas mixture with 8.8% O₂, which has a P_{O₂} (66 torr) equivalent to 21,000-22,000 ft high altitude, was chosen for subsequent experiments.

Unanesthetized rabbits were exposed to the following gas mixtures: air; 20% O₂ + 5.6% CO₂; 8.8% O₂; 8.8% O₂ + 5.6% CO₂; and 8.8% O₂ + 10% CO₂. Mixtures other than air were prepared in nitrogen. For convenience, gas mixtures containing 8.8% O₂ are termed "hypoxic" or "hypoxic + CO₂." Pre- and air exposed animals served as normal and experimental controls respectively.

A single experiment consisted of exposing a rabbit to one of the above gas mixtures for up to 5 hrs. Arterial blood samples were collected after 30 min and 5 hrs exposure. In addition, 2 hr samples were drawn with the hypoxic and hypoxic + 5.6 CO₂ exposures.

Blood samples were analyzed for ESF, pH, P_{O₂}, Hct, Hb, oxygen saturation (S_{O₂}), adenosine 5'-triphosphate (ATP), adenosine 5'-diphosphate (ADP), 2,3-DPG, lactate, and pyruvate.

3) A final series of experiments were conducted to determine if the magnitude of the initial ESF response to hypoxia is dependent on a continuous hypoxic stimulus.

Rabbits were exposed to hypoxia (8.8% O₂) for 30 min or 2 hrs, or to hypoxia + 5.6% CO₂ for 2 hrs. These exposures were immediately followed by an exposure to room air until a total combined exposure time of 5 hrs had elapsed. Arterial blood samples were drawn after the 5 hr exposure period for the determination of plasma ESF concentrations. These ESF concentrations were compared to those of rabbits exposed continuously to

hypoxia or hypoxia + 5.6% CO₂ for 5 hrs (see above).

Animal preparation.

An unanesthetized rabbit was placed in a standard restraining device which allowed for a normal crouched position with minimal discomfort (see Plate I). One ear was shaved and the blood vessels dilated with xylene. The auricular artery was cannulated with a #19 gauge needle attached to polyethylene tubing (inside diameter .055"). Two hundred units sodium heparin/kg body weight were injected to prevent blood clotting during the experiment. Rapid blood flow into a 2 ml syringe containing 1,000 units sodium heparin/ml normal saline indicated unobstructed cannulation. The rabbit was transferred to the exposure chamber and the cannula attached to an external 2-way stopcock. The cannula was cleared periodically with heparinized saline (100 units/ml saline) throughout the experiment.

Exposure chamber.

The exposure chamber used for all experiments (including the exposure to air) was a lucite box with 4.8 cu ft capacity. A standard gas tank (200 cu ft) with adjustable valving fed gas into one end of the chamber. The gas outlet from the chamber entered a Wet Test Meter (Precision Scientific Co., Chicago, Illinois) and the flow rate was adjusted to 30 cu ft per hr, giving approximately six gas exchanges per hr. Thirty min were allowed for chamber equilibration before the timed experiment began. Pre-mixed gases (Pacific Oxygen Co., Oakland, Calif.) were re-analyzed to ensure correct composition; only gas mixtures within + 0.2% of the required percentage composition were used.

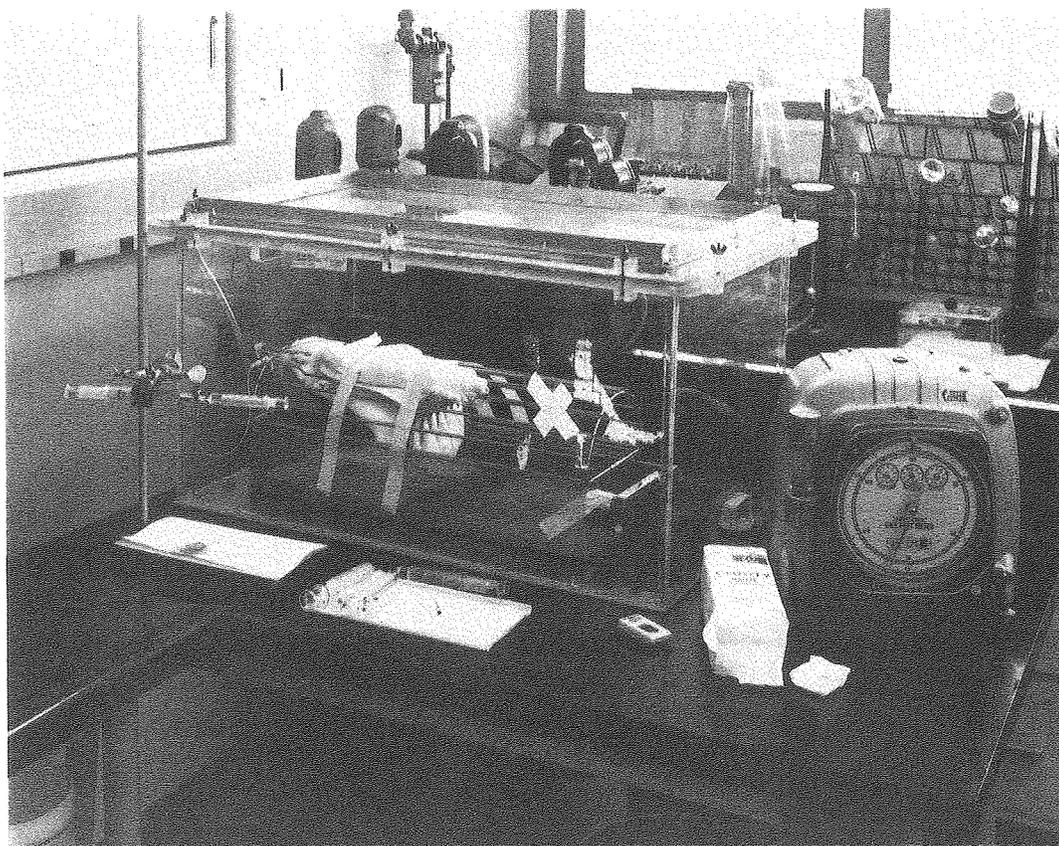


PLATE I

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LUCITE EXPOSURE CHAMBER AND APPARATUS FOR SAMPLING
THE BLOOD OF RABBITS EXPOSED TO VARIOUS GAS MIXTURES.

Samplings.

Blood from the auricular artery was used for all assays and analyses. The blood was drawn anaerobically without stasis into a glass syringe via the external 2-way stopcock; a glass bead and sodium heparin filled the dead space. The blood sample was mixed with the glass bead by gentle inversion and rotation immediately after sampling.

In order to minimize the effects of blood loss, no more than two blood samples were drawn from an animal during a single experiment. At no time did a single blood sample exceed 7% by weight of the total blood volume (10-15 ml blood per sample); this estimate was based on a total blood volume of 55.6 - 57.3 ml blood/kg body weight (Kozma et al., 1974).

To ascertain whether or not measured parameters were affected by previous sampling, a number of checks were made:

- 1) Only one sample was taken in some experiments for each gas mixture and time period.

- 2) Sampling time periods were overlapped for different experiments with the same gas mixtures, e.g., pre-exposure and 30 min, 30 min and 5 hrs, etc.

- 3) In some experiments, very small blood volumes were drawn (< 5 ml) for the first sample.

Comparison of samples from one time period with the same gas mixture showed no significant differences in the measured parameters as a result of prior sampling.

Measurements and assays.

Assays were completed within five days from the time of sampling; the bioassay for ESF within three months. Reproducibility was checked periodically by doing the assays in duplicate. All reagents and enzyme preparations were purchased from Sigma Chemical Co., Saint Louis, Missouri. Spectrophotometric determinations were made with the DU-2 spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) and ^{59}Fe radioactivity measured with a gamma well scintillation counter (Nuclear-Chicago). A PDP-8 computer (Digital Equipment Corp., Maynard, Massachusetts) was used for most of the statistical computations. The standard student's t-test was used to determine the significance levels of all measurements.

(1) Measurements.

(a) P_{O_2} , P_{CO_2} , pH and S_{O_2} : For the determination of P_{O_2} and P_{CO_2} , non-heparinized capillary tubes, 150 lambda capacity (Radiometer, Copenhagen, Denmark), were filled with whole blood immediately after sampling. Blood was drawn into standard heparinized hematocrit tubes for the determination of pH and S_{O_2} . All tubes were sealed with plasticine after insertion of a small piece of steel wire; the blood was magnetically mixed, and the tubes were submerged in an ice bath until used (within 2 hrs from the time of sampling). Prior to measurement, each tube was brought to room temperature, magnetically re-mixed, and the ends discarded to prevent ambient air contamination. Measurements of the P_{O_2} , P_{CO_2} and pH were made in triplicate with the Blood Micro System, Type BMS 3b (Radiometer), and values were monitored with the Digital Acid-Base Analyzer, Type PHM 72C (Radiometer). Electrodes were calibrated at 37°C to a sensitivity of ± 0.1 torr for P_{O_2} and P_{CO_2} , and $\pm .005$ Units for pH. The S_{O_2} was measured in quadruplicate

by the freeze-thaw method (Siggaard-Andersen et al., 1962) with the dual-beam Type OSML Oxygen Saturation Meter (Radiometer) calibrated with normal rabbit blood.

(b) Hct and Hb: The Hct was measured by the standard micromethod and the Hb by the cyanmethemoglobin colorimetric method; both measurements were done in duplicate.

(c) Hemoglobin-oxygen affinity (P_{50}): A 5 ml sample of whole-blood was equilibrated at two P_{O_2} levels with the IL 237 Tonometer (Instrumentation Laboratory, Inc., Lexington, Massachusetts).

These P_{O_2} levels were adjusted to fall above and below the estimated $P_{50(7.4)}$ within an S_{O_2} range of 35-65%. This range was within the linear segment of the oxygen dissociation curve. The P_{O_2} was controlled with the IL 208-01 Gas-mixing Module and IL 208-02 Oxygen Monitor (Instrumentation Laboratory, Inc.). The system was operated at 37° C, P_{CO_2} at 30 torr, and with fully water-saturated gases. Complete equilibration of the blood with the pre-selected gas mixtures was checked independently by measuring the P_{O_2} and P_{CO_2} . Blood samples for the determination of P_{O_2} , P_{CO_2} , pH and S_{O_2} were drawn into capillary tubes from the tonometer and immediately measured as described above. The P_{O_2} was corrected to pH 7.4 and the $P_{50(7.4)}$ was interpolated from the plot of the corrected P_{O_2} versus S_{O_2} ; for this purpose, a Bohr factor of $\frac{\Delta \text{Log } P_{O_2}}{\Delta \text{pH}} = -0.45$, given by Hilpert et al., (1963) for rabbit blood, was used. The $P_{50(i.v.)}$ was calculated from the $P_{50(7.4)}$ by correcting to the pH and Base Excess (BE) observed in vivo. The equation:

$$\begin{aligned} \text{Log } P_{50(i.v.)} = & \text{Log } [26.6 + 0.5 (\text{MCHC}-33) + 0.69 (\text{DPG}-14.5)] \\ & + 0.0013 \text{ BE} + 0.48 (7.4-\text{pH}) + 0.024 (T-37) \end{aligned} \quad (1)$$

given by Bellingham et al., (1971) for calculation of the $P_{50(i.v.)}$ for human blood was modified to give an approximation of the $P_{50(i.v.)}$ of rabbit blood:

$$\text{Log } P_{50(i.v.)} = \text{Log } P_{50(7.4)} + 0.0013 \text{ BE} + 0.45 (7.4 - \text{pH}). \quad (2)$$

This simplified expression for the $P_{50(i.v.)}$ eliminates the MCHC and 2,3-DPG terms since they are already incorporated in the experimentally determined $P_{50(7.4)}$; it is assumed that these factors do not change significantly during the brief period of tonometry. The equation of Bellingham et al., (1971) (equation 1) was further modified in equation 2 by replacing the Bohr factor for the human (-0.48) with that for the rabbit (-0.45) and by deleting the temperature factor. It was assumed that the rabbit's body temperature does not change significantly during exposure to the isobaric gas mixtures carried out at room temperature (23° C).

The BE was estimated with the Blood Gas Calculator of Severinghaus (1966) by using the in vivo pH, P_{CO_2} , and Hb values.

(2) Assays.

(a) Erythropoietin (ESF): The plasma concentrations of ESF were estimated by the procedure developed by Fogh (1966) and modified by Schooley and Mahlmann (1972a). Assay animals were female LAF₁/JAX mice made plethoric by exposure to CO for two weeks. One week after removal from the CO chamber, the mice were injected subcutaneously with a one ml sample of test plasma. Samples expected to contain high concentrations of ESF were first diluted with normal rabbit plasma; in all cases, a total of one ml plasma was injected. Three to eight mice were used for the determination of the ESF concentration of each test sample. Fifty-six hrs later, ⁵⁹Fe (New England

Nuclear Corp., Boston, Massachusetts), 0.5 μ Ci in 0.2 ml citrate-normal saline, was injected intravenously. Seventy-two hrs later, a 0.5 ml blood sample was taken by cardiac puncture; blood samples with Hcts below 55% were discarded. Conversion to Units of ESF was made by relating the 72 hr percent ^{59}Fe uptake (assuming 7% blood volume by weight) to a standard curve prepared using the International Reference Preparation (I.R.P.).

(b) Lactate and pyruvate: Determination of lactate and pyruvate concentrations by enzymatic analyses were made on whole-blood as described in the 1968 Sigma Technical Bulletins Nos. 726-UV and 826-UV (Sigma Chemical Co., Saint Louis, Missouri). A protein-free filtrate in 8% perchloric acid was prepared at the time of sampling and stored at 0 to 4^o C until assayed (within 5 days).

Enzymes: Lactate dehydrogenase.

The degree of average whole-body tissue hypoxia, expressed by "excess lactate" (XL) accumulation, was calculated, with minor modification, according to the equation developed by Huckabee (1958):

$$XL = (L_n - L_o) - (P_n - P_o)(L_o/P_o)$$

where L_o and P_o are the control (pre-exposure) arterial blood lactate and pyruvate concentrations; L_n and P_n are the concentrations under experimental conditions. Since not every experiment gave both a pre-exposure and experimental value for each experimental time period, the mean pre-exposure values for lactate and pyruvate were substituted for the individual pre-exposure values in the above equation.

(c) Intraerythrocytic organic phosphates:

2,3-DPG - The enzymatic assay procedure described in Sigma Technical

Bulletin No. 35-UV (1971) was used to determine 2,3-DPG concentrations.

A filtrate from hemolyzed whole-blood was prepared in 8% TCA at the time of sampling and stored at 0 to 4^o C until assayed (within 5 days).

Enzymes: 2,3-DPG phosphatase + phosphoglycerate kinase + glyceraldehyde phosphate dehydrogenase.

ATP - The enzymatic assay procedure described in Sigma Technical Bulletin No. 366 (1973) was used to determine ATP concentrations. Whole-blood was hemolyzed and proteins precipitated in 12% TCA at the time of sampling.

Uncentrifuged samples were stored at -20^oC until assayed (within 5 days).

Enzymes: Phosphoglycerate kinase + glyceraldehyde phosphate dehydrogenase.

ADP - The enzymatic assay system used for the determination of ADP was developed by Sigma Chemical Co. (personal communication); it is a modification of the system described by Lowry et al., (1964). Filtrate preparation and storage conditions were the same as for ATP (described above).

Enzymes: Pyruvate kinase + lactate dehydrogenase.

RESULTS

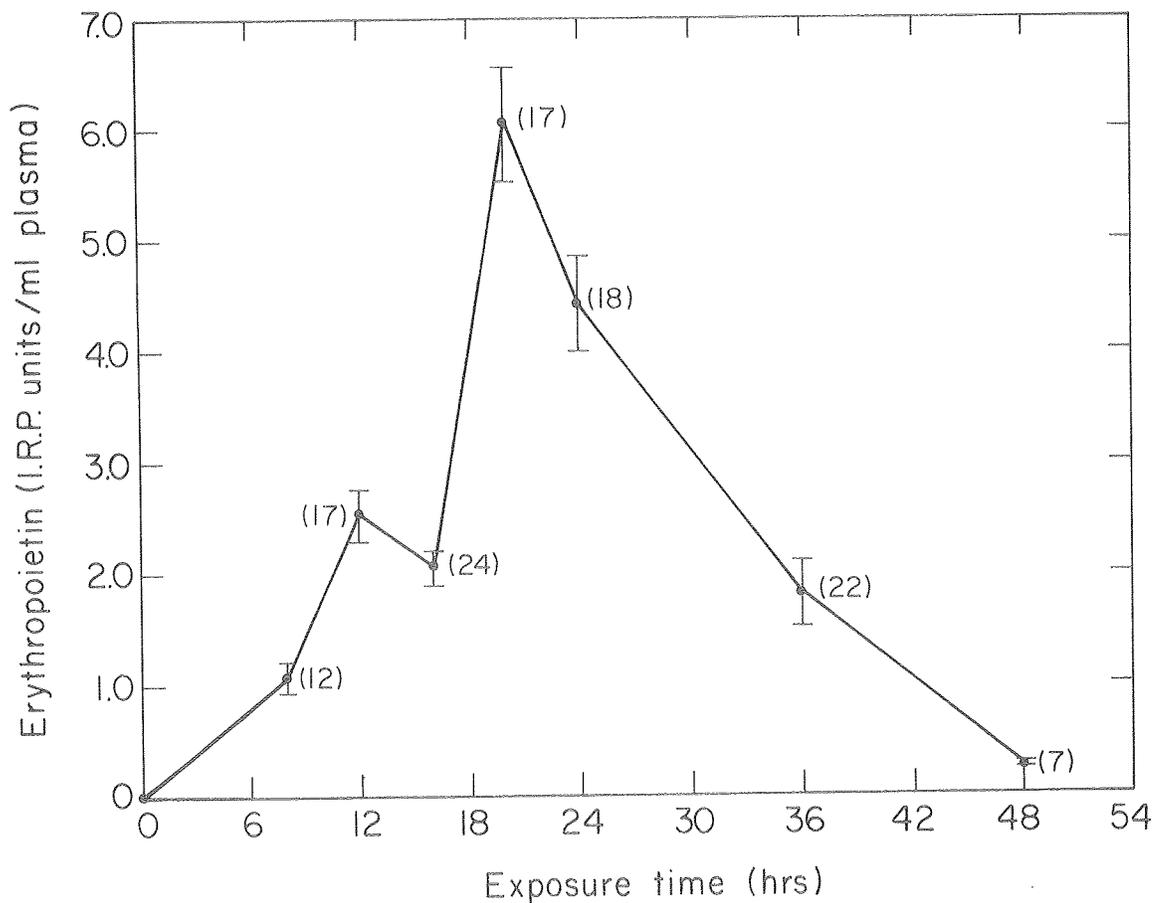
Some of the data to be discussed in detail are presented in both tabular and graphic form for additional clarity. Graphs include the standard error wherever such values do not obscure the graphic presentation; in cases of overlapping values for a single time period the largest standard error is given.

Erythropoietin (ESF).

Fig. 1 shows the plasma ESF levels of rabbits exposed to 21,000 - 22,000 ft simulated altitude for up to 48 hrs. A rapid increase in the ESF concentration to 1.07 ± 0.14 (\pm SEM) I.R.P. Units/ml plasma was observed after 8 hrs exposure. A peak ESF level of 6.05 ± 0.54 Units/ml plasma was reached after 20 hrs exposure followed by a rapid decline to 0.04 ± 0.02 Units/ml plasma after 48 hrs. Though the rabbits seemed to hyperventilate excessively, they appeared to be healthy during the 48 hrs exposure to this severe simulated altitude.

Exposure of rabbits to 8.8% O_2 under isobaric conditions for 2 hrs resulted in a small increase in plasma ESF levels (0.04 ± 0.01 Units/ml plasma); by 5 hrs the ESF level had risen to 0.69 ± 0.06 Units/ml plasma. Fig. 2 shows that the addition of CO_2 to this hypoxic gas mixture reduced the ESF response very significantly; after 2 hrs exposure with 5.6% CO_2 added no ESF was detectable and after 5 hrs the ESF response was reduced by 83% or 91% with 5.6% or 10% CO_2 added.

In the experiments reported in Table I, the plasma ESF levels were measured in rabbits exposed briefly to 8.8% O_2 or 8.8% O_2 + 5.6% CO_2 followed by exposure to room air for a total exposure time of 5 hrs; these ESF levels



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FIG. 1. ERYTHROPOIETIN (ESF) CONCENTRATIONS IN ARTERIAL RABBIT PLASMA DURING 48 HRS EXPOSURE TO A SIMULATED ALTITUDE OF 21,000 - 22,000 FT. UNITS OF ESF WERE DETERMINED BY REFERENCE TO A STANDARD I.R.P. RESPONSE CURVE AFTER MEASUREMENT OF THE 72 HR ^{59}Fe UPTAKE IN PLETHORIC LAF₁/JAX FEMALE MICE. SIX - TWELVE MICE WERE TESTED PER RABBIT. TWO RABBITS WERE TESTED AFTER 8, 12, 16, 20, 24, or 36 HRS ELAPSED EXPOSURE TIME; ONLY ONE RABBIT WAS TESTED AT 48 HRS. THE NUMBER IN PARENTHESIS REFERS TO THE NUMBER OF ASSAY MICE TESTED. VALUES ARE PRESENTED AS THE MEAN \pm SEM, BASED ON THE TOTAL NUMBER OF ASSAY MICE.

were compared to those of rabbits continuously exposed to hypoxia or hypoxia + 5.6% CO₂ for 5 hrs. A 30 min hypoxic exposure caused no measurable increase in plasma ESF levels in rabbits bled 4-1/2 hrs later. Rabbits exposed to 2 hrs of hypoxia and bled 3 hrs later had much lower ESF levels (0.07 ± 0.01 Units/ml plasma) than those exposed to hypoxia for 5 hrs (0.69 ± 0.06 Units/ml plasma). Similarly, the single rabbit exposed to hypoxia + 5.6% CO₂ for 2 hrs and bled 3 hrs later, had a lower ESF titer (0.04 ± 0.00) than those exposed to hypoxia + 5.6% CO₂ for 5 hrs (0.12 ± 0.01 Units/ml plasma).

Lactate, pyruvate, and calculated "excess lactate" (XL).

The mean whole-blood lactate, pyruvate, and calculated XL values are presented in Table II; changes in XL are also shown graphically in Fig. 3. The pre-exposure lactate and pyruvate concentrations were 1.85 and 0.131 μ moles/ml blood respectively. After 5 hrs exposure to "air" the concentrations increased to 5.25 and 0.308 μ moles/ml blood. The hypoxic exposure caused a very dramatic increase in both lactate and pyruvate; by 5 hrs lactate levels were up to 11.00 μ moles/ml blood and pyruvate 0.398 μ moles/ml blood. Rabbits exposed for 5 hrs to normoxia or hypoxia with CO₂ added showed only minor increases in lactate and pyruvate.

The increase in lactate relative to pyruvate was similar for each gas mixture except for the 8.8% O₂. The hypoxic exposure resulted in a more rapid increase in lactate than pyruvate, causing a very significant rise in XL to 5.34 μ moles/ml blood after 5 hrs exposure. Addition of 5.6% or 10% CO₂ to the hypoxic gas mixture apparently alleviated tissue hypoxia since XL did not accumulate during the 5 hr exposure period.

TABLE II

LACTATE, PYRUVATE AND CALCULATED EXCESS LACTATE (XL) CONCENTRATIONS OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂.

Gas mixture flowing through chamber	EXPOSURE TIME										
	30 Min.			2 Hrs.			5 Hrs.			Excess Lactate	
	Lactate	Pyruvate	Excess* Lactate	Lactate	Pyruvate	Excess Lactate	Lactate	Pyruvate	Pyruvate		
AIR	1.22±.20** (N = 7)	.079±.018 (N = 7)	.10±.08 (N = 7)	5.25±.66† (N = 7)	.308±.028† (N = 7)	.89±.48 (N = 7)	2.98±.46 (N = 6)	.210±.042 (N = 6)	.03±.36 (N = 7)	.308±.028† (N = 7)	.89±.48 (N = 7)
20% O ₂ + 5.6% CO ₂	1.18±.16 (N = 6)	.049±.009 (N = 6)	.16±.18 (N = 7)	6.36±.60† (N = 11)	.309±.023† (N = 11)	1.95±.27† (N = 10)	2.98±.46 (N = 6)	.210±.042 (N = 6)	.03±.36 (N = 7)	.398±.017† (N = 8)	5.34±1.05† (N = 8)
8.8% O ₂	3.76±.42† (N = 7)	.231±.013† (N = 8)	.53±.28 (N = 8)	1.69±.28 (N = 8)	.091±.008 (N = 9)	.40±.22 (N = 8)	11.00±1.12† (N = 8)	.398±.017† (N = 8)	5.34±1.05† (N = 8)	.217±.030 (N = 9)	.85±.64 (N = 8)
8.8% O ₂ + 5.6% CO ₂	.95±.11 (N = 9)	.056±.007 (N = 9)	.16±.08 (N = 9)	1.69±.28 (N = 8)	.091±.008 (N = 9)	.40±.22 (N = 8)	2.52±.33 (N = 6)	.217±.030 (N = 9)	.85±.64 (N = 8)	.217±.030 (N = 9)	.85±.64 (N = 8)
8.8% O ₂ + 10% CO ₂	.90±.07 (N = 8)	.059±.007 (N = 9)	.15±.07 (N = 9)	3.36±.63 (N = 8)	.174±.027 (N = 8)	.89±.33 (N = 8)	3.36±.63 (N = 8)	.174±.027 (N = 8)	.89±.33 (N = 8)	.174±.027 (N = 8)	.89±.33 (N = 8)

*Excess Lactate = $L_2 - (P_2 \times \frac{L_1}{P_1})$;
 $\frac{\text{Pre-Exposure lactate (L}_1\text{)}}{\text{Pre-Exposure pyruvate (P}_1\text{)}} = 14.20$

**Mean ± 1 SEM

†P < .001

Lactate	Pyruvate
(μmoles/ml whole blood)	(μmoles/ml whole blood)
1.85±.19 (N = 22)	.131±.014 (N = 20)

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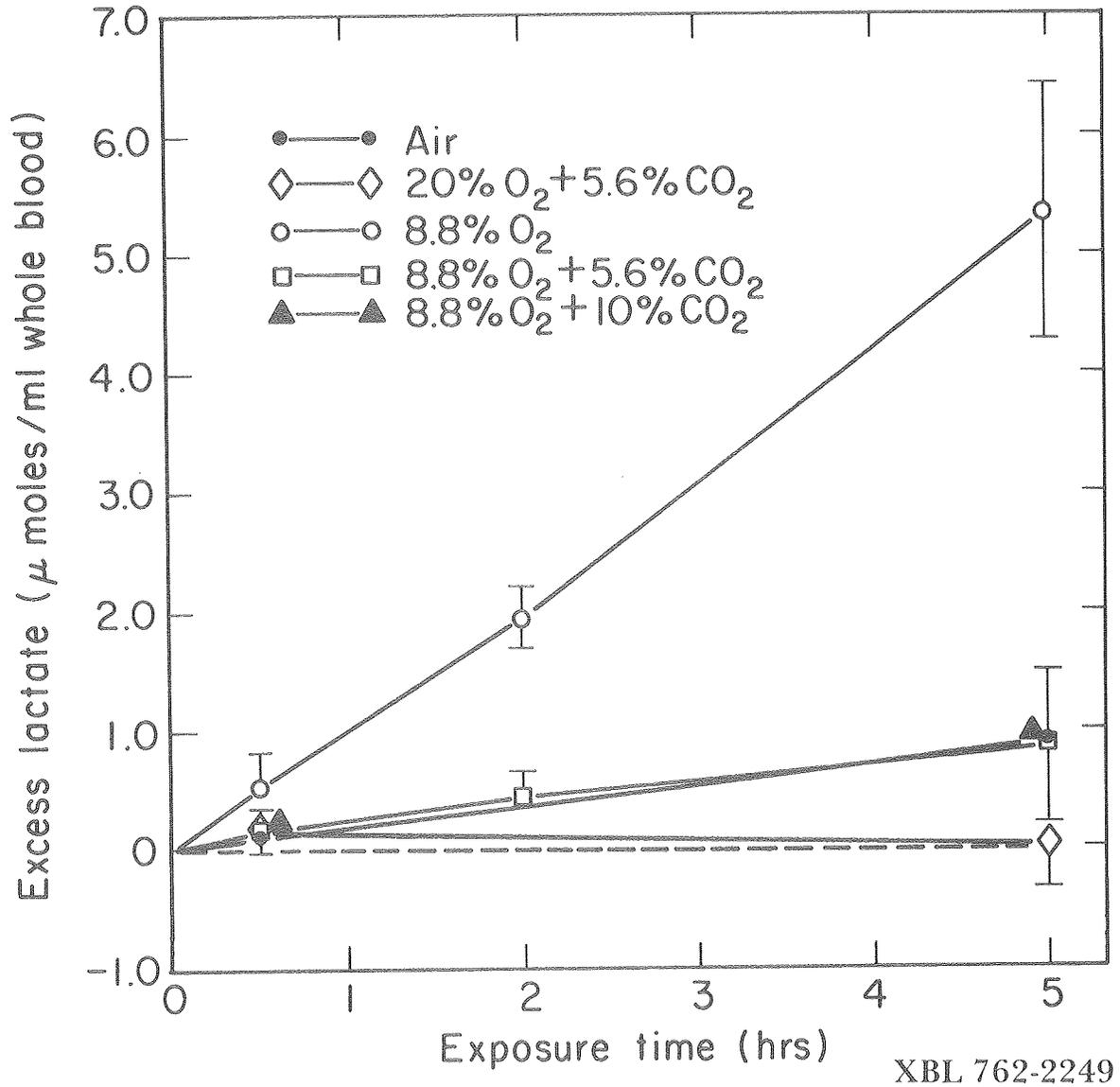


FIG. 3. CALCULATED EXCESS LACTATE OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂. DASHED LINE (---) REPRESENTS ZERO XL. VALUES ARE PRESENTED AS THE MEAN \pm 1 SEM.

Partial pressure of oxygen (P_{O_2}) and oxygen saturation (S_{O_2}).

The mean P_{O_2} and S_{O_2} values for arterial whole-blood are presented in Table III and Fig. 4. The pre-exposure P_{O_2} was 75.6 torr with an S_{O_2} of 94.4%. Addition of 5.6% CO_2 to the normoxic gas mixture increased the P_{O_2} 15% above the pre-exposure value with no major shift in the S_{O_2} . With exposure to hypoxia, the P_{O_2} decreased to 33.8 torr by 30 min and remained at that level up to 5 hrs exposure; the S_{O_2} gradually decreased from 71.9% at 30 min to 63.7% at 5 hrs. Addition of 5.6% or 10% CO_2 to the hypoxic gas mixture partially prevented this decrease in P_{O_2} , resulting in P_{O_2} values 22% or 34% respectively above the value for hypoxia alone. The S_{O_2} values for the exposures to hypoxia + CO_2 were indistinguishable at 30 min from the value for hypoxia alone; at 5 hrs the S_{O_2} value for the exposure to hypoxia + 5.6% CO_2 was the same as the 5 hr hypoxic value, while that for hypoxic + 10% CO_2 was slightly higher.

Acid-base balance.

The mean values for the pH, P_{CO_2} and BE of arterial whole-blood are presented in Table IV; the changes in pH and P_{CO_2} are also shown graphically in Fig. 5. Exposure to air for 5 hrs caused a slight respiratory alkalosis; the pH increased to 7.50 and the P_{CO_2} decreased to 22.0 torr from the pre-exposure values of 7.45 and 33.4 torr respectively. A pronounced respiratory alkalosis was induced after 30 min by the hypoxic exposure (pH, 7.65; P_{CO_2} , 17.1 torr); partial recovery of the pH to 7.51 was observed after 5 hrs with no changes in P_{CO_2} . This recovery probably occurred as a result of increases in fixed acids as reflected by a BE of -8.5 m Eq/l. As seen in Table II, elevated lactate (approximately 9 m Eq/l) apparently accounted for this phenomenon. Addition of 5.6% CO_2 to the hypoxic gas mixture effectively blocked the respiratory alkalosis and decrease in BE. Hypoxia + 10% CO_2

TABLE III

PERCENT OXYGEN SATURATION (S_{O_2}) AND PARTIAL PRESSURE OF OXYGEN (P_{O_2}) OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO_2 .

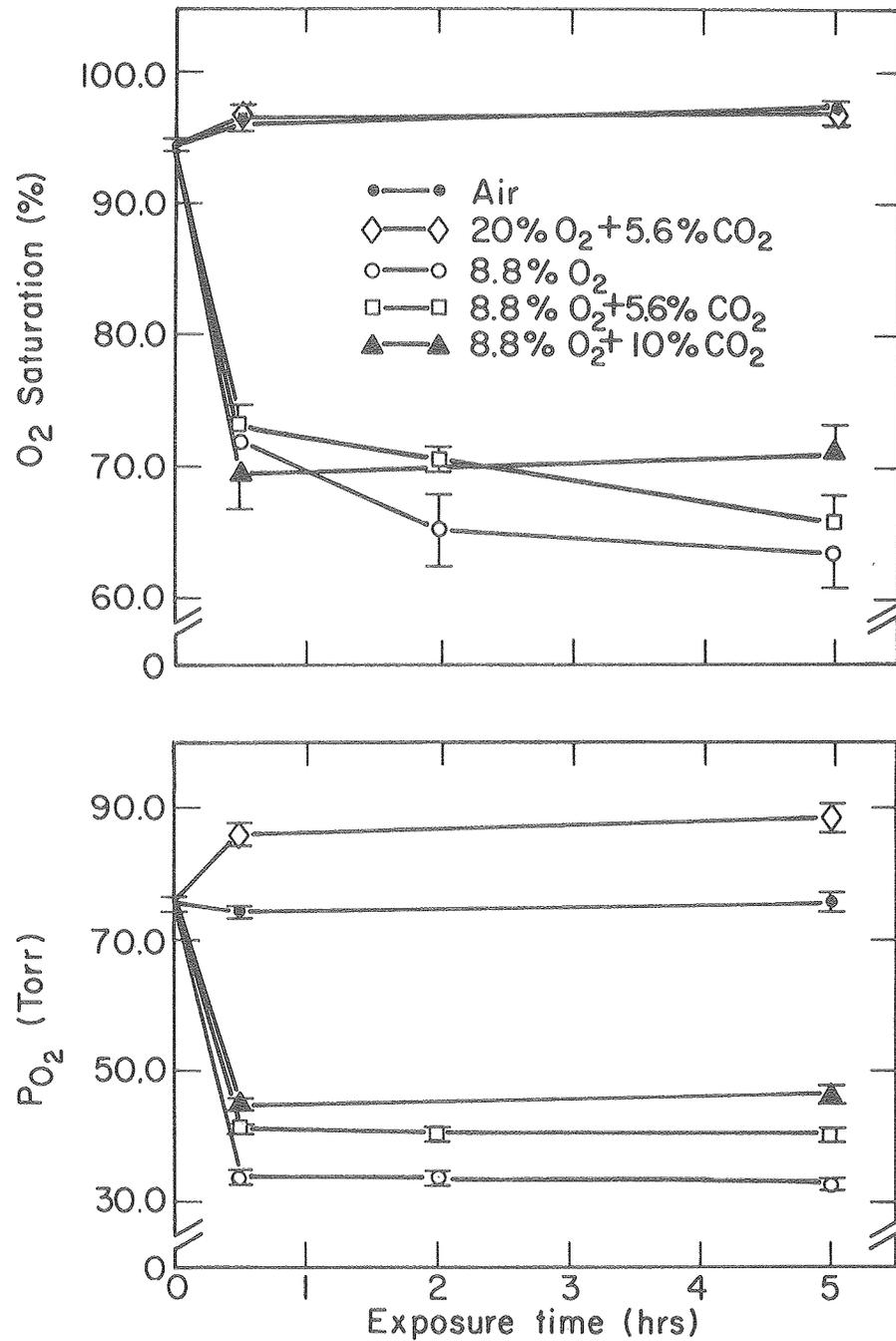
Gas Mixture flowing Through chamber	EXPOSURE TIME					
	30 Min.		2 Hrs.		5 Hrs.	
	% O ₂ Sat	P _{O₂} (Torr)	% O ₂ Sat.	P _{O₂} (Torr)	% O ₂ Sat.	P _{O₂} (Torr)
AIR	96.1±.6* (N = 7)	74.2±1.0 (N = 7)			97.5±.5 (N = 7)	75.7±1.4 (N = 7)
20%O ₂ + 5.6% CO ₂	96.4±.4 (N = 7)	86.1±1.7† (N = 7)			97.3±.6 (N = 7)	88.5±2.2† (N = 7)
8.8% O ₂	71.9±2.9† (N = 8)	33.8±1.1† (N = 8)	65.3±2.7† (N = 12)	33.8±1.0† (N = 12)	63.7±2.7† (N = 8)	32.9±.8† (N = 8)
8.8% O ₂ + 5.6% CO ₂	73.0±1.9† (N = 9)	41.1±.7† (N = 9)	70.6±1.0† (N = 8)	40.4±1.1† (N = 8)	66.6±1.5† (N = 8)	40.2±.9† (N = 8)
8.8% O ₂ + 10% CO ₂	69.5±2.6† (N = 9)	44.7±1.0† (N = 9)			71.4±2.0† (N = 8)	46.4±1.3† (N = 8)

*Mean ± 1 SEM

†p < .001

Pre-Exposure	% O ₂ Sat.	P _{O₂} (Torr)
	94.4±.5 (N = 19)	75.6±1.0 (N = 19)

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FIG. 4. S_{O_2} (ABOVE) AND P_{O_2} (BELOW) OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO_2 . VALUES ARE PRESENTED AS THE MEAN \pm 1 SEM.

TABLE IV
 ACID-BASE BALANCE OF ARTERIAL WHOLE-BLOOD OF RABBITS
 DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂.

Gas mixture flowing through chamber	EXPOSURE TIME											
	30 min.				2 Hrs.				5 Hrs.			
	pH	P _{CO₂} (Torr)	Calculated Base Excess (m Eq/Liter)		pH	P _{CO₂} (Torr)	Calculated Base Excess (m Eq/Liter)		pH	P _{CO₂} (Torr)	Calculated Base Excess (m Eq/Liter)	
AIR	7.46±.01* (N = 7)	30.2±.6 (N = 7)	-1.1±0.3 (N = 7)		7.50±.01 (N = 7)	22.0±1.7† (N = 7)	-4.2±1.1† (N = 7)		7.50±.01 (N = 7)	22.0±1.7† (N = 7)	-4.2±1.1† (N = 7)	
20% O ₂ + 5.6% CO ₂	7.37±.01† (N = 7)	43.0±.5† (N = 7)	-0.8±0.4 (N = 7)		7.36±.02† (N = 7)	41.5±.7† (N = 6)	-1.5±0.9 (N = 7)		7.36±.02† (N = 7)	41.5±.7† (N = 6)	-1.5±0.9 (N = 7)	
8.8% O ₂	7.65±.02† (N = 8)	17.1±.6† (N = 8)	-0.3±1.2 (N = 8)		7.58±.01† (N = 11)	16.9±.9† (N = 11)	-3.7±1.0† (N = 11)		7.51±.03 (N = 8)	15.9±.8† (N = 8)	-8.5±0.9† (N = 8)	
8.8% O ₂ + 5.6% CO ₂	7.42±.01 (N = 9)	41.8±.6† (N = 9)	2.1±0.4 (N = 9)		7.39±.01† (N = 9)	42.8±1.0† (N = 9)	0.5±0.6 (N = 9)		7.37±.01 (N = 9)	41.4±.9† (N = 9)	-1.6±0.6 (N = 9)	
8.8% O ₂ + 10% CO ₂	7.30±.01† (N = 9)	68.6±.8† (N = 9)	4.5±0.9† (N = 9)		7.28±.01 (N = 8)	70.8±1.4† (N = 8)	4.1±1.2† (N = 8)		7.28±.01 (N = 8)	70.8±1.4† (N = 8)	4.1±1.2† (N = 8)	
Pre-Exposure	pH	P _{CO₂} (Torr)	Calculated Base Excess (m Eq/Liter)									
	7.45±.01 (N = 19)	33.4±.7 (N = 19)	-0.1±0.5 (N = 19)									

*Mean ± 1 SEM

† P < .001

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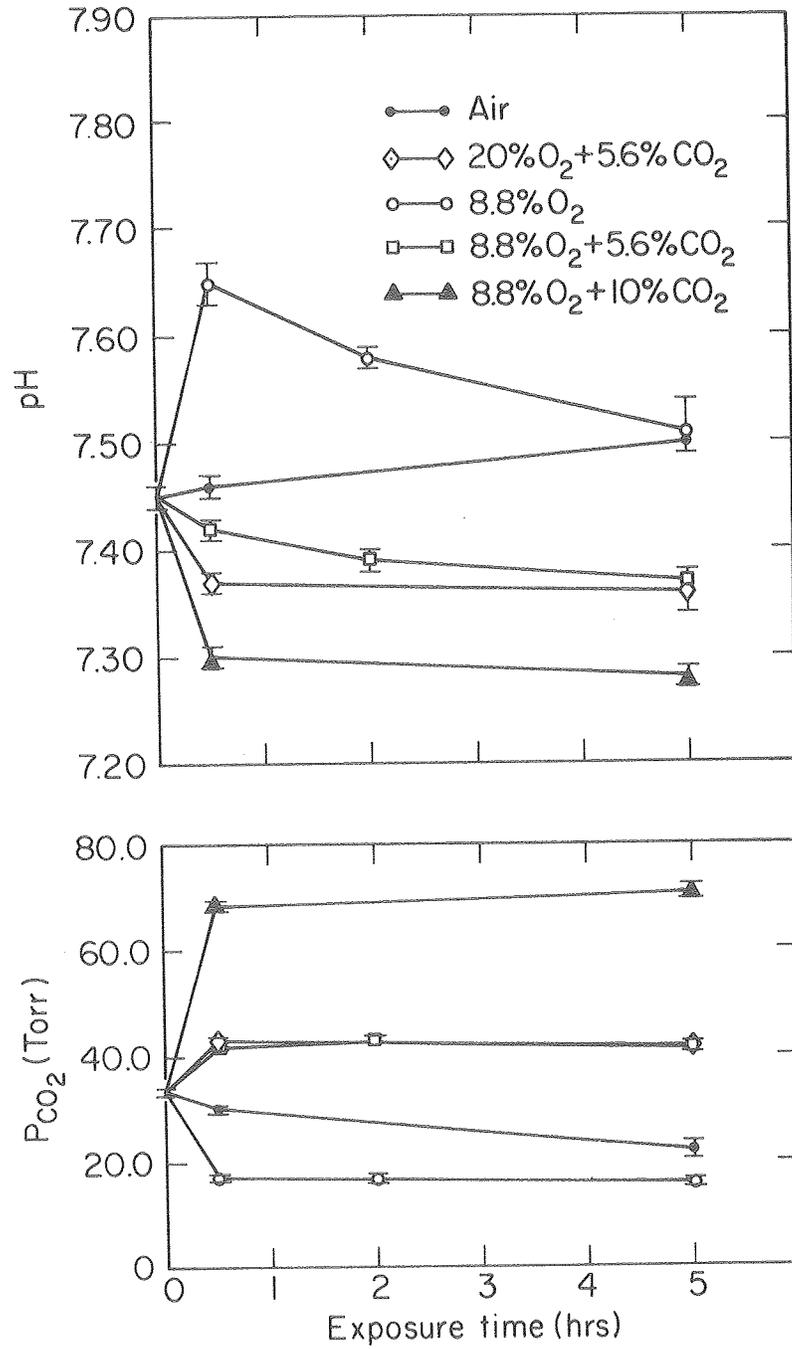
TABLE V
 HEMATOLOGICAL PARAMETERS OF ARTERIAL BLOOD OF RABBITS
 DURING EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂.

Gas mixture flowing through chamber	EXPOSURE TIME											
	30 min.				2 Hrs.				5 Hrs.			
	Hematocrit (%)	g Hemoglobin 100 ml blood	MCHC g Hemoglobin 100 ml cells		Hematocrit (%)	g Hemoglobin 100 ml blood	MCHC g Hemoglobin 100 ml cells		Hematocrit (%)	g Hemoglobin 100 ml blood	MCHC g Hemoglobin 100 ml cells	
AIR	39.1±1.7* (N = 7)	13.6±.3 (N = 7)	33.4±.4 (N = 7)					38.7±.8 (N = 7)	13.0±.4 (N = 7)	33.6±.5 (N = 7)		
20% O ₂ + 5.6% CO ₂	41.4±.8 (N = 7)	14.0±.3 (N = 7)	33.8±.3 (N = 7)					39.4±1.0 (N = 7)	13.6±.4 (N = 7)	34.4±.2 (N = 7)		
8.8% O ₂	39.7±1.3 (N = 9)	13.6±.5 (N = 9)	34.3±.3 (N = 9)		38.6±1.1 (N = 13)	13.0±.4 (N = 13)	33.7±.3 (N = 13)	36.6±1.5 (N = 8)	12.7±.5 (N = 8)	34.7±.3† (N = 8)		
8.8% O ₂ 5.6% CO ₂	40.1±.7 (N = 9)	13.4±.2 (N = 9)	33.4±.2 (N = 9)		40.2±.7 (N = 9)	13.4±.3 (N = 9)	33.2±.2 (N = 9)	38.4±.9 (N = 9)	12.8±.3 (N = 9)	33.4±.2 (N = 9)		
8.8% O ₂ + 10% CO ₂	39.5±1.1 (N = 9)	12.8±.4 (N = 9)	32.4±.4 (N = 9)					38.3±1.1 (N = 9)	12.3±.4 (N = 9)	32.1±.5† (N = 9)		
Pre-Exposure	40.3±.5 (N = 27)	13.5±.2 (N = 27)	33.4±.1 (N = 27)									

*Mean ± 1 SEM

†P<.001

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XBL 762-2357

FIG. 5. PH (ABOVE) AND P_{CO₂} (BELOW) OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂. VALUES ARE PRESENTED AS THE MEAN ± 1 SEM.

caused a respiratory acidosis by 30 min (pH, 7.30; P_{CO_2} , 68.6 torr) which persisted up to 5 hrs.

Hematological parameters.

Estimates of the arterial hematological parameters are presented in Table V. The pre-exposure Hct was 40.3% with values decreasing slightly with time for all gas mixtures; a minimum value of 36.6% was observed after 5 hrs exposure to hypoxia. The mean pre-exposure Hb concentration was 13.5 g%. A small decrease was observed at the end of 5 hrs exposure with all gas mixtures except normoxia + 5.6% CO_2 . The mean pre-exposure MCHC was 33.48 g Hb/100 ml packed cells. No MCHC changes were observed with exposure to air or hypoxia + 5.6% CO_2 . A 3% MCHC increase occurred by the end of 5 hrs exposure to normoxia + 5.6% CO_2 . With hypoxia alone the MCHC increased 4% by 5 hrs. The opposite effect was observed with exposure to hypoxia + 10% CO_2 , where the MCHC decreased 4% after 5 hrs.

The affinity of Hb for oxygen (P_{50}).

The mean P_{50} values are presented in Table VI and Fig. 6. The pre-exposure $P_{50(7.4)}$ was 31.9 torr. No significant change in the $P_{50(7.4)}$ was observed with exposure to air. After 2 hrs of exposure to hypoxia, a large increase in $P_{50(7.4)}$ to 34.2 torr was observed; this increased $P_{50(7.4)}$ was maintained for the remaining 3 hrs of the 5 hr exposure. Addition of CO_2 to the hypoxic gas mixture caused a very significant decrease of the $P_{50(7.4)}$ to about 30 torr within 30 min; no further decrease was observed after 5 hrs exposure. The pre-exposure $P_{50(i.v.)}$ was 30.8 torr. A significant decrease of the $P_{50(i.v.)}$ to 27.2 torr occurred after 5 hrs exposure to air. Exposure to normoxia or hypoxia with 5.6% CO_2 added

TABLE VI

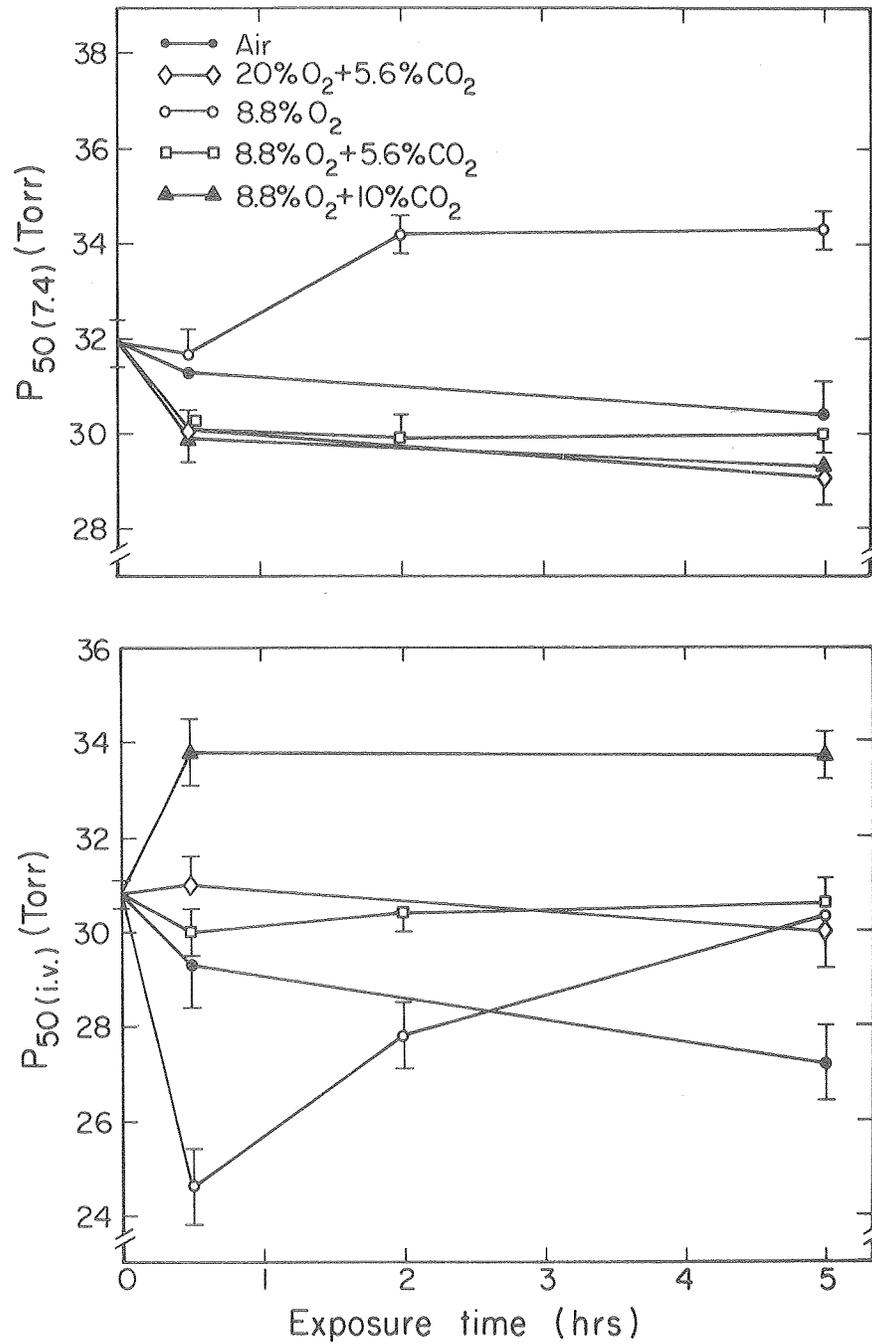
THE IN VITRO AND IN VIVO P₅₀ OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂.

Gas mixture flowing through chamber	EXPOSURE TIME					
	30 min.		2 Hrs.		5 Hrs.	
	P ₅₀ (7.4) (Torr)	P ₅₀ (i.v.) (Torr)	P ₅₀ (7.4) (Torr)	P ₅₀ (i.v.) (Torr)	P ₅₀ (7.4) (Torr)	P ₅₀ (i.v.) (Torr)
AIR	31.3 ± 0.8* (N = 6)	29.3 ± 0.9 (N = 6)	—	—	30.4 ± 0.7 (N = 6)	27.2 ± 0.8† (N = 6)
20% O ₂ 5.6% CO ₂	30.1 ± 0.5 (N = 7)	31.0 ± 0.6 (N = 7)	—	—	29.1 ± 0.6 (N = 6)	30.0 ± 0.8 (N = 6)
8.8% O ₂	31.7 ± 0.5 (N = 8)	24.6 ± 0.8† (N = 7)	34.2 ± 0.4 (N = 7)	27.8 ± 0.7† (N = 7)	34.3 ± 0.4 (N = 6)	30.3 ± 0.8 (N = 6)
8.8% O ₂ 5.6% CO ₂	30.1 ± 0.6 (N = 6)	30.0 ± 0.5 (N = 6)	29.9 ± 0.5 (N = 6)	30.4 ± 0.4 (N = 6)	30.0 ± 0.4 (N = 7)	30.6 ± 0.5 (N = 6)
8.8% O ₂ 10% CO ₂	29.9 ± 0.5 (N = 9)	33.8 ± 0.7† (N = 9)	—	—	29.3 ± 0.4 (N = 8)	33.7 ± 0.5† (N = 7)
Pre-Exposure	P ₅₀ (7.4) (Torr)	P ₅₀ (i.v.) (Torr)				
	31.9 ± 0.5 (N = 14)	30.8 ± 0.3 (N = 13)				

*Mean ± 1 SEM

†p < .001

XBL785-3175



XBL 766-3060

FIG. 6. THE IN VITRO (ABOVE) AND IN VIVO (BELOW) P_{50} OF ARTERIAL WHOLE-BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO_2 . VALUES ARE PRESENTED AS THE MEAN \pm 1 SEM.

did not cause a significant shift of the $P_{50(i.v.)}$ from the pre-exposure value. A dramatic decrease in the $P_{50(i.v.)}$ to 26.6 torr occurred after a 30 min hypoxic exposure; after 2 hrs a partial recovery to 27.8 torr occurred; at 5 hrs the $P_{50(i.v.)}$ was indistinguishable from the pre-exposure value of 30.8 torr. Exposure to hypoxia + 10% CO_2 for 30 min caused a rapid increase in the $P_{50(i.v.)}$ to 33.8 torr which was maintained for the 5 hr exposure period.

Organic phosphates.

Table VII gives the mean intraerythrocytic 2,3-DPG, ATP, and ADP concentrations; the changes in ADP are presented graphically in Fig. 7. The mean pre-exposure concentration of 2,3-DPG was 27.58 μ moles/g Hb. A transient decrease at 30 min followed by a recovery approaching the pre-exposure level after 5 hrs exposure was observed with air and hypoxia + 5.6% CO_2 . Exposure to hypoxia caused a gradual increase in 2,3-DPG (statistically insignificant) to 28.98 μ moles /g Hb after 5 hrs. The pre-exposure concentration of ATP was 5.16 μ moles/g Hb. The only significant shift in the ATP concentration occurred with exposure to normoxia + 5.6% CO_2 ; this exposure caused a decrease to 3.73 and 3.59 μ moles/g Hb after 30 min and 5 hrs respectively. The pre-exposure concentration of ADP was 2.83 μ moles/g Hb. After 5 hrs exposure to air the ADP concentration increased to 4.52 μ moles/g Hb. With exposure to hypoxia, a very rapid increase in ADP was observed by 30 min, with a continued rise to 6.82 μ moles/g Hb after 5 hrs. There was an initial small decline followed by a return to the pre-exposure value after 5 hrs in either normoxia + 5.6% CO_2 or hypoxia + CO_2 .

TABLE VII

INTRAEERYTHROCYTIC ORGANIC PHOSPHATE CONCENTRATIONS OF ARTERIAL BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂.

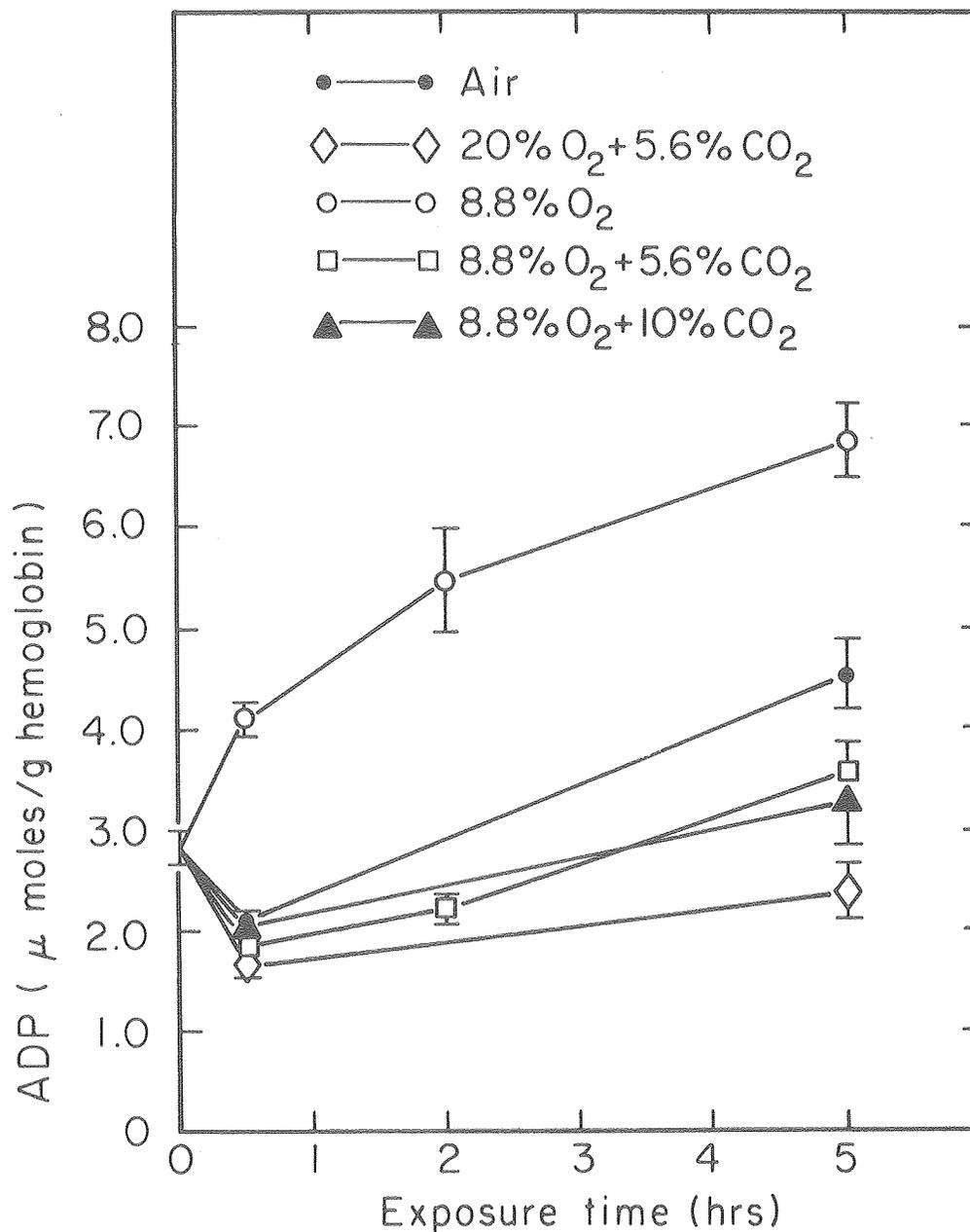
Gas mixture flowing through chamber	EXPOSURE TIME					
	30 min.		2 Hrs.		5 Hrs.	
	ATP	ADP	2,3-DPG	ATP	ADP	2,3-DPG
AIR	4.93±.27* (N = 7)	2.09±.10 (N = 7)	25.85±1.22 (N = 7)	4.67±.36 (N = 7)	4.52±.35† (N = 7)	27.39±1.32 (N = 7)
20% O ₂ + 5.6% CO ₂	3.73±.45 (N = 7)	1.66±.13† (N = 6)	26.72±.86 (N = 7)	3.59±.58 (N = 7)	2.36±.26 (N = 6)	25.48±.80 (N = 7)
8.8% O ₂	4.35±.30 (N = 9)	4.10±.17† (N = 8)	27.67±.83 (N = 9)	4.45±.28 (N = 12)	5.46±.53† (N = 6)	28.98±.93 (N = 8)
8.8% O ₂ + 5.6% CO ₂	4.62±.21 (N = 9)	1.83±.12† (N = 9)	25.44±.69 (N = 9)	4.56±.23 (N = 9)	2.16±.15 (N = 9)	26.14±.51 (N = 9)
8.8% O ₂ + 10% CO ₂	4.39±.22 (N = 9)	2.03±.09† (N = 9)	25.97±.89 (N = 8)	4.93±.25 (N = 8)	3.25±.43 (N = 8)	24.76±.84 (N = 7)

*Mean ± 1 SEM

† P < .001

Pre-Exposure	ATP	ADP	2,3-DPG
	(μmoles/g Hemoglobin)		
	5.16±.17 (N = 24)	2.83±.17 (N = 9)	27.58±.42 (N = 24)

XBL778-3736



XBL778-3740

FIG. 7. INTRAERYTHROCYTIC ADP CONCENTRATIONS OF ARTERIAL BLOOD OF RABBITS DURING 5 HRS EXPOSURE TO HYPOXIA WITH AND WITHOUT CO₂. VALUES ARE PRESENTED AS THE MEAN \pm 1 SEM.

DISCUSSION

In order to interrelate the observations made in this study it is necessary to make occasional use of assumptions bordering on oversimplification. An attempt will be made to make the reader aware of such potential pitfalls in the process of applying a meaningful interpretation to the data.

Suppression of ESF production in response to alleviated tissue hypoxia.

Evidence has been presented which shows that the ESF response observed in the unanesthetized rabbit during a 5 hr exposure to 8.8% O_2 is almost completely blocked by the addition of 5.6% or 10% CO_2 to the hypoxic gas mixture. The effectiveness of this ESF suppression by CO_2 is comparable to that reported by Schooley and Mahlmann (1975) in the rat, where addition of 10% CO_2 to 10% O_2 caused a 95% reduction in serum ESF titers after a 4 hr exposure.

Inadequate tissue oxygenation is generally accepted as the primary cause for increased plasma ESF titers, although neither the specific renal nor the extra-renal ESF producing tissues have been identified. By implication, therefore, alleviated tissue hypoxia is the probable cause for the ESF inhibition observed when CO_2 is added to hypoxic gas mixtures. In order to implicate a decrease in tissue hypoxia as the cause for the ESF suppression, it is necessary to demonstrate that tissue hypoxia is reduced when CO_2 is added to the hypoxic gas mixture.

The measurement used to give an estimate of the degree of hypoxia to which the body tissues are subjected is XL. Unlike whole-blood lactate,

XL is highly correlated with oxygen debt and expresses lactate accumulation above that expected from an increase in overall glycolysis; it thus serves as an indicator of anaerobic metabolism (Huckabee, 1958). The anesthetized dog and unanesthetized human accumulate XL within 30 min when exposed to 10% O₂ (Huckabee, 1958). The degree of environmental hypoxia necessary to cause XL accumulation in the unanesthetized rat during the first 30 min of exposure appears to vary from 6.6% O₂ (Altland et al., 1967) to 13% O₂ (Lewis et al., 1973). Similar studies have not been reported for the rabbit.

The present study shows that XL is detectable in the unanesthetized rabbit after 30 min exposure to an environment containing 8.8% O₂. This shift to anaerobic metabolism is maintained with continued exposure to hypoxia for up to 5 hrs. Addition of CO₂ to the hypoxic environment prevents this shift to anaerobic metabolism and XL does not accumulate.

If one were to simply look at the lactate levels, the air exposure would give a false impression of an existing hypoxic state since after 5 hrs exposure the lactate concentration increased from 1.85 to 5.25 μ moles/ml blood. Since the concentration of pyruvate increased at the same rate as lactate, resulting in no XL, it can be concluded that the rate of glycolysis has increased with no detectable oxygen deprivation.

The increases in lactate and pyruvate in the air control rabbits are probably an indirect reflection of stress. In order to circumvent the suppressive effects of anesthesia on pulmonary ventilation, cardiac output, etc., all experiments were performed without anesthesia. Consequently, some stress was caused by the experimental manipulations, i.e., restraint, cannulation, and placement in the exposure chamber for up to 5 hrs. (an attempt was made, without success, to pre-condition the rabbits to the

experimental conditions). This stress results in the gradual development of a minor respiratory alkalosis. This alkalosis, already initiated during the pre-exposure period (pH, 7.45; P_{CO_2} , 33.4 torr), continues through 5 hrs exposure to air (pH, 7.50; P_{CO_2} , 22.0 torr). The increase in lactate and pyruvate observed in the air exposed rabbits and the fraction of the increase in lactate which is proportional to that of pyruvate in rabbits exposed to hypoxia, is probably due to an overall increase in the rate of glycolysis stimulated by alkalosis (Mansour, 1963; Minakami and Yoshikawa, 1966; Ui, 1966; Rorth and Nygaard, 1971; Jacey and Schaefer, 1972).

The exact oxygen concentration at which a tissue can be declared "hypoxic" is dependent on the metabolic requirements of that tissue. These requirements are met until the end-capillary P_{O_2} declines to levels which compromise oxygen diffusion to the tissues. It must be stressed that whole-blood XL represents the average XL accumulation of all body tissues and that each tissue and organ has a different micro-circulation and metabolic rate with different oxygen requirements.

It is not possible to conclude that XL accumulation is a reflection of oxygen availability to the renal cells which respond to hypoxia by increasing ESF production. However, it is possible to state that the addition of CO_2 to 8.8% O_2 alleviates average tissue hypoxia as reflected by XL accumulation and that this is the probable cause of the suppression of ESF production.

The effect of CO_2 on tissue oxygenation during exposure to hypoxia.

Tissue hypoxia can be alleviated through enhanced oxygen delivery to the tissues and/or decreased tissue metabolism. It is beyond the scope of this study to speculate about the effects of CO_2 on cellular metabolism

or aspects of the oxygen delivery system other than those related to the arterial P_{O_2} and P_{50} .

If the added CO_2 relieves tissue hypoxia by way of altering the arterial P_{O_2} and/or P_{50} , these parameters could be expected to differ from those of animals exposed to hypoxia alone before differences in ESF titers are observed. Miller (1975) showed that the leftward $P_{50(i.v.)}$ shift precedes the increase in ESF titers by several hours in the human and rat. This is probably because inadequate tissue oxygenation is a stimulus for de novo ESF synthesis, not merely a trigger for an all-or-none release of stored hormone (Giger, 1968; Schooley and Mahlmann, 1972a); hence the time-lag between the hypoxic stimulus and measurable increases in ESF.

Results of the present study with rabbits suggest that increases in plasma ESF concentrations in response to hypoxia are also at least in part, the result of de novo ESF synthesis. When rabbits were exposed to hypoxia or hypoxia + 5.6% CO_2 for 2 hrs and bled 3 hrs later, ESF titers were considerably lower than those of animals exposed continuously for 5 hrs to the same gas mixture. This indicates that even though a sufficient degree of tissue hypoxia has been reached at the end of 2 hrs exposure to trigger ESF production, the hypoxic stimulus must be maintained in order to cause further production of ESF. When one looks at the ESF levels after a 2 hr exposure, animals exposed to hypoxia alone already show measurable increases in ESF titers; those exposed to hypoxia + 5.6% CO_2 show no increases in ESF levels. Though not conclusive, the data suggest that ESF production is initiated prior to 2 hrs exposure to hypoxia or hypoxia + CO_2 . With hypoxia alone tissue hypoxia is more severe than with hypoxia + CO_2 . This difference in oxygen availability between animals exposed to

hypoxia or hypoxia + CO₂, as reflected by differences in plasma ESF concentrations after 2 hrs exposure should be operational prior to 2 hrs exposure. For this reason, the following discussion will focus primarily on the changes in the arterial P_{0₂} and P₅₀ observed at the end of the 30 min exposure period.

It is established that brief inhalation of gas mixtures either low in oxygen or high in carbon dioxide stimulates pulmonary ventilation; a combination of low oxygen with high CO₂ enhances this effect (Slonim and Hamilton, 1971). Such hyperventilation raises the arterial P_{0₂} unless other variables affecting pulmonary gas exchange counteract this effect. The results of the present study are very similar to those reported by Pepelko (1972) for the rat, with respect to augmentation of the arterial P_{0₂} by CO₂ during exposure to a hypoxic environment: The higher the % CO₂ admixture, the higher the arterial P_{0₂} (at inspired P_{CO₂} levels of 90 torr or below).

In spite of the fact that the arterial P_{0₂} is very significantly higher in rabbits breathing 8.8% O₂ + CO₂ than in those breathing 8.8% O₂ alone, the arterial S_{0₂} remains constant at about 70% after 30 min exposure to hypoxia with or without CO₂ added. This might appear paradoxical because one would expect the arterial S_{0₂} to be elevated in animals with an elevated arterial P_{0₂} if changes in the P_{50(i.v.)} are not considered. The addition of CO₂ to the hypoxic gas mixture not only raises the arterial P_{0₂} but also causes a relative increase in the P_{50(i.v.)}. The results of the present study suggest that the increase in arterial P_{0₂} balances this decrease in Hb-O₂ affinity, resulting in a constant arterial S_{0₂}. Thus, despite the decrease of the Hb-O₂ affinity, oxygen loading in the lungs is maintained by the simultaneous increase in pulmonary ventilation. On the other hand, this same decrease in Hb-O₂ affinity facilitates oxygen unloading to the

tissues. The net effect is that the tissue hypoxia occurring in rabbits breathing 8.8% O_2 alone is partially alleviated when CO_2 is added to the gas mixture. Consequently, XL does not accumulate, and ESF production that would be expected during hypoxia, is almost abolished.

The contribution of increased pulmonary ventilation and shifts in the P_{50} to tissue oxygenation during hypoxia.

The ability of an animal to compensate for environmental hypoxia is in part a function of the limitations imposed by that animal's oxygen requirements and normal oxygen delivery system. Metcalfe and Dhinsa (1970) compared the ways in which different animal species meet their respective oxygen requirements under normal conditions. In comparing the human and rabbit, they found that the three-fold higher oxygen consumption per kg body weight of the rabbit was compensated for by a doubling of the cardiac output relative to body size and by an increase in the arteriovenous oxygen difference by 1-1/2 fold, the latter being a function of the higher rabbit P_{50} . The authors ask the hypothetical question, "Will the rabbit's response to stress be limited by its high basal cardiac output?" On a speculative basis, if the upper limit of the cardiac output is approached during exposure to severe hypoxia, it is likely that the rabbit would be less able to rely on a further increase in cardiac output, hence more on ventilatory and/or P_{50} compensation than the human. This does not imply that the human cannot effectively utilize his ventilatory reserve to compensate for hypoxia. In fact, Lenfant et al., (1971) concluded from their results with humans at altitude that "Of all the changes in oxygen transport parameters occurring at an altitude of 4,509 m, the increase in ventilation is that with the greatest effect in improving O_2 supply." Both the

rapidity of onset and the degree of respiratory alkalosis observed in the present experiments with 8.8% O_2 suggest that respiratory compensation plays a major role in partially off-setting low ambient P_{O_2} levels. Addition of CO_2 to the hypoxic gas mixture demonstrates even more clearly the rabbit's ability to utilize its ventilatory reserve.

It is not possible to make a general statement about the benefits of a right- or left-shifted P_{50} during hypoxia. Whether tissue oxygenation is enhanced or decreased by a P_{50} shift depends on the normal position of the P_{50} of the species in question and on the severity of the hypoxia to which that species is subjected. Hall (1966) found that the critical P_{O_2} (the lowest oxygen tension at which an animal can extract oxygen from its environment) among 18 rodent species alone varied from 8 torr to 36 torr, and that a strong positive correlation exists between the critical P_{O_2} and the normal $P_{50(7.4)}$; the $P_{50(7.4)}$ ranged from 22 torr to 53 torr. Baumann et al., (1971) calculated that the right-shifted $P_{50(7.4)}$, in response to increases in 2,3-DPG after 36 hrs at 4,000 m altitude, did not improve oxygen delivery in the rat, yet increased oxygen extraction 27% in the guinea pig. These investigators concluded that:

"Due to the slope of the ODC, a decrease in oxygen affinity increases oxygen delivery to the tissues only as long as arterial oxygen saturation remains above 70%. Since even with a normal oxygen affinity arterial oxygen saturation at 4,000 m is below 70% in the rat, the further decrease in oxygen affinity...cannot increase oxygen extraction.

Since guinea pigs have a considerably higher oxygen affinity, their arterial oxygen saturation at 4,000 m remains above 70% even after the moderate decrease of the oxygen affinity in the hypoxic animals."

The above conclusions are based on the $P_{50(7.4)}$ instead of the $P_{50(i.v.)}$ and should be accepted with caution. However, the principle is applicable to the $P_{50(i.v.)}$. In the present study with rabbits, the arterial S_{O_2}

is maintained at about 70% when CO_2 is added to the hypoxic gas mixture. This occurs despite the relative decrease in Hb- O_2 affinity because increased ventilation raises the arterial P_{O_2} . In the absence of this increased ventilation, the benefits of the relatively right-shifted $\text{P}_{50(\text{i.v.})}$ would be very much open to question since the arterial S_{O_2} would drop below 70%. This deduction is supported by the findings of Rand et al., (1973). Rabbits, 2,3-DPG loaded or depleted, with $\text{P}_{50(\text{i.v.})}$'s of 37 torr and 24 torr respectively, were exposed to graded hypoxia (21% to 5% inspired oxygen) at fixed ventilation. No significant differences between the two groups were observed in implanted polarographic electrode measurements of brain, renal cortex and sacrospinalis muscle tissues.

Some evidence suggests that an increase, rather than a decrease, in Hb- O_2 affinity is advantageous during very severe hypoxic stress. Turek et al., (1976) chronically administered sodium cyanate (NaOCN) to rats prior to a severe hypoxic exposure, thereby decreasing the $\text{P}_{50(7.4)}$ from 35.9 torr to 21.9 torr. When the NaOCN treated animals were exposed to 8% or 5.6% O_2 they had a higher venous P_{O_2} and a greater arteriovenous oxygen difference than the untreated controls similarly exposed. The authors concluded that the NaOCN treated rats were in better "shape" during severe hypoxia, as a result of the left-shifted ODC. Using a similar experimental protocol, Eaton et al., (1974) studied the survival rate of rats at extreme simulated altitude (28,000 ft) during a 90 min exposure period. All the NaOCN treated rats ($\text{P}_{50(7.4)} = 21.0$ torr) survived even though they had a depressed heart rate; the untreated controls ($\text{P}_{50(7.4)} = 37.3$ torr) had a survival rate of only 20%. The difference in $\text{P}_{50(7.4)}$, instead of the $\text{P}_{50(\text{i.v.})}$, was used by the authors to explain the difference in survival rate. This appears to be a valid

explanation if the pH measurements of Turek *et al.*, (1976) are applicable, since the $P_{50(i.v.)}$ difference between the two groups would have been even greater because the pH of the NaOCN group was significantly higher than that of the controls.

The underlying causes for the P_{50} shifts.

The common practice of using the $P_{50(7.4)}$ instead of the $P_{50(i.v.)}$ considerably increases the possibility of misinterpreting the effects of P_{50} shifts on oxygen delivery to the tissues. This is clearly demonstrated by the results of the present experiments where a 30 min exposure to 8.8% O_2 caused a dramatic decrease of the $P_{50(i.v.)}$, yet the $P_{50(7.4)}$ remained constant. However, when 10% CO_2 was added, the $P_{50(i.v.)}$ increased while the $P_{50(7.4)}$ decreased.

In order to isolate the factors which cause these shifts in P_{50} and contribute to the differences between the $P_{50(i.v.)}$ and $P_{50(7.4)}$, it is useful to examine in some detail the equation developed by Bellingham *et al.*, (1971) based on data for the human:

$$\begin{aligned} \text{Log } P_{50(i.v.)} = & \text{Log } [26.6 + 0.5(\text{MCHC}-33) + 0.69(\text{DPG}-14.5)] \\ & + 0.0013 \text{ BE} + 0.48(7.4-\text{pH}) + 0.024(\text{T}-37). \end{aligned} \quad (1)$$

A similar equation has not been derived for the rabbit but a first approximation can be made by substituting some of the known and presently determined values for the rabbit into equation (1):

$$\begin{aligned} \text{Log } P_{50(i.v.)} = & \text{Log } [31.9 + 0.5(\text{MCHC}-33.4) + 0.69(\text{DPG}-27.6)] \\ & + 0.0013 \text{ BE} + 0.45(7.4-\text{pH}) + 0.024(\text{T}-37) \end{aligned} \quad (2)$$

where,

31.9 (torr) = the mean pre-exposure $P_{50(7.4)}$

33.4 (g Hb/100 ml cells) = the mean pre-exposure MCHC

27.6 (μ moles/g Hb) = the mean pre-exposure 2,3-DPG concentration

0.45 ($\Delta \text{Log } P_{O_2} / \Delta \text{pH}$) = the Bohr factor for the rabbit

37°C = the normal body temperature of the rabbit.

By replacing the pre-exposure $P_{50(7.4)}$ with the experimental $P_{50(7.4)}$ in equation (2), the MCHC and 2,3-DPG terms drop out since they are an inherent part of the experimentally determined $P_{50(7.4)}$:

$$\begin{aligned} \text{Log } P_{50(i.v.)} &= \text{Log } P_{50(7.4)} + 0.0013 \text{ BE} + 0.45(7.4-\text{pH}) \\ &+ 0.024(T-37). \end{aligned} \quad (3)$$

Equation (4), derived from equation (3) by deleting the temperature factor, is the expression used for the determination of the $P_{50(i.v.)}$ in the present experiments:

$$\text{Log } P_{50(i.v.)} = \text{Log } P_{50(7.4)} + 0.0013 \text{ BE} + 0.45(7.4-\text{pH}). \quad (4)$$

It was not determined whether changes in body temperature were completely circumvented by exposing the rabbits to isobaric gas mixtures at room temperature (23°C). Changes in body temperature may still occur as a result of hyperventilation and shifts in circulation in response to the gas mixtures used. Decreases in temperature have been observed in rabbits breathing air plus CO₂ (Stupfel, 1974). Rabbits exposed to air plus 5%, 10%, or 20% CO₂ lowered their temperature (skin, muscular, and rectal) by 0.8°C,

1.3°C, and 2.3°C per hour exposure, respectively. If hypoxia-hypercapnia were to cause similar decreases in temperature, the $P_{50(i.v.)}$ of the present study, especially the 5 hr exposure to 8.8% O_2 + 10% CO_2 , would contain a significant positive error. However, the error would be negligible during the first 30 min of exposure, the time period most relevant to this study.

The BE term in equation (4) is an estimate of the influence of carbamino formation on the Hb- O_2 affinity (Naeraa et al., 1966). This term has been retained "as is" from equation (1) since there is no compelling evidence which suggests that the human and rabbit differ significantly with respect to the influence of carbamino formation on the $P_{50(i.v.)}$. It is common practice to estimate the BE of experimental animals by the use of alignment nomograms based on the data from human blood: Singer and Hastings, 1948; Siggaard-Andersen, 1963; Severinghaus, 1966. Estimates of the BE in the present experiments with the rabbit were made with the alignment nomogram of Severinghaus, 1966. These estimates are probably not strictly correct since the normal buffer line for the rabbit is probably slightly different from that of the human and the formation of metabolic acids and deoxyhemoglobin alters the capacity of the blood to buffer bicarbonate changes in response to large fluctuations in the P_{CO_2} of the blood (Pepelko, 1972).

The validity of using equation (4) for the determination of the $P_{50(i.v.)}$ can be checked independently by application of a derivation of the Hill equation. Lichtman et al., (1976) developed an expression for the determination of the venous $P_{50(i.v.)}$ which is independent of factors other than the in vivo P_{O_2} and S_{O_2} values:

$$P_{50(i.v.)} = \text{Antilog} [(\text{Log } 1/k)/2.7] \quad (5)$$

where,

$$1/k = [\text{Antilog}(2.7 \text{ Log } P_{O_2})] [(100 - S_{O_2})/S_{O_2}]$$

Using the P_{O_2} and S_{O_2} values obtained from arterial instead of venous blood in the present experiments with the 8.8% O_2 , 8.8% O_2 + 5.6% CO_2 , and 8.8% O_2 + 10% CO_2 still satisfies the primary requirement of the equation, that the S_{O_2} be low enough to fall on the linear segment of the ODC. Estimates of the $P_{50(i.v.)}$, derived through equation (5), do not deviate significantly from the values determined by the application of equation (4) for all three gas mixtures and time periods. Furthermore, if the ODC given by Bartels (1971) for the rabbit is shifted to fit the $P_{50(i.v.)}$ values derived through equation (4), the S_{O_2} values extrapolated from the measured P_{O_2} values agree with the measured S_{O_2} values. Unless there are errors which coincidentally offset each other, equation (4) appears to give a good estimate of the rabbit $P_{50(i.v.)}$.

Changes in $P_{50(7.4)}$ reflect only the influence of changes in MCHC and 2,3-DPG on $P_{50(i.v.)}$ shifts (see equation 2) since BE, pH, and temperature are held constant by definition. Therefore, under some experimental conditions when the pH deviates from 7.4 and/or the BE from zero, the $P_{50(i.v.)}$ and $P_{50(7.4)}$ need not be similar. In the present study, this is most clearly demonstrated by the P_{50} changes observed with the exposure to 8.8% O_2 . After 30 min the $P_{50(i.v.)}$ decreased very significantly, yet the $P_{50(7.4)}$ remained constant. Since the change in BE was insignificant, this shift was caused by the increase in pH. After 2 hrs the $P_{50(i.v.)}$

had partially recovered. This was caused by the partial recovery of the pH as a result of fixed acid accumulation and by the same factors which contribute to the rapid increase in the $P_{50(7.4)}$, e.g., increases in MCHC and 2,3-DPG. After 5 hrs the $P_{50(i.v.)}$ had recovered to the pre-exposure level primarily as a result of the continued decline of the pH.

In all experiments, the pH-independent effect of CO_2 on the $P_{50(i.v.)}$, quantitated by the BE, was relatively small. This is exemplified by the results of the 5 hr exposure to 8.8% O_2 . This exposure caused the largest shift in BE (-8.5 m eq/l). The $P_{50(7.4)}$ was 34.3 torr and the $P_{50(i.v.)}$ 30.3 torr. This 4 torr difference is comprised of the BE and pH components which contribute approximately 0.8 torr and 3.2 torr, respectively.

The $P_{50(i.v.)}$ is an expression of the P_{50} which incorporates the influence of all major elements which are known to affect the Hb- O_2 affinity. The $P_{50(7.4)}$ is a useful measurement for determining the influence of changes in the MCHC and organic phosphates on the $P_{50(i.v.)}$. The difference between the pre-exposure $P_{50(7.4)}$ and $P_{50(7.4)}$ expresses the influence of these two factors on the $P_{50(7.4)}$:

$$P_{50(7.4)} = \text{Pre-exposure } P_{50(7.4)} + 0.5(\text{MCHC}-33.4) + 0.69(\text{DPG}-27.6). \quad (6)$$

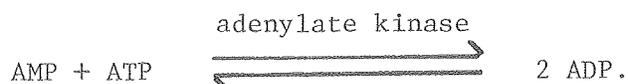
The above regression of the MCHC and 2,3-DPG on the $P_{50(7.4)}$, as determined by Bellingham *et al.*, (1971) for the human, is most likely not identical to that of the rabbit. However, for a first approximation Bunn (1971) has presented evidence which tentatively supports the validity of using the

regression of 2,3-DPG on the $P_{50(7.4)}$ of the human for that of the rabbit. He demonstrated in vitro (Hb solutions) that animal species with very low normal intraerythrocytic 2,3-DPG levels, e.g., cat, goat, sheep, etc., do not appreciably shift their P_{50} in response to increases in 2,3-DPG. In contrast, species with high normal 2,3-DPG levels, like the human and the rabbit, not only increase their P_{50} with increases in 2,3-DPG, but do so with similar slope characteristics. A rough estimate of the P_{50} shift per μ mole 2,3-DPG/g Hb for the rabbit, can be derived from the data of Rand et al., (1973). Rabbits, 2,3-DPG loaded and depleted, 27-37 and 11-14 μ moles/g Hb respectively, had $P_{50(i.v.)}$'s of 37 and 24 torr. Using the estimated means of these 2,3-DPG ranges (means not given by the authors) and assuming linearity, the P_{50} increases approximately 0.65 torr per μ mole 2,3-DPG/g Hb; a value which is comparable to the value of 0.69 for the human.

Unlike the other gas mixtures, the hypoxic exposure caused a significant increase in the $P_{50(7.4)}$. If the pre-exposure $P_{50(7.4)}$ and the 2 or 5 hr values for 2,3-DPG and MCHC are substituted into equation (6), the calculated $P_{50(7.4)}$ is lower than the measured $P_{50(7.4)}$ by 1.9 and 0.8 torr respectively. This discrepancy could be due to the increase in intraerythrocytic ADP concentrations which are not incorporated into equation (6). Due to the high 2,3-DPG levels in the red cells, the contribution of ATP, and especially ADP, to shifts in the P_{50} are frequently ignored. The present experiments show that the concentrations of ATP and 2,3-DPG do not change significantly during the hypoxic exposure, while that of ADP does, measuring 4 μ moles per g Hb above the pre-exposure values after 5 hrs. This increase is almost three times greater than that of 2,3-DPG. Benesch and Benesch (1967)

showed that at a concentration of 5×10^{-3} M the effect of the di- and triphosphates on the Hb- O_2 affinity are similar. On the other hand, Perutz (1970) states that "ATP, ADP and AMP have binding constants with haemoglobin lower than DPG by one, two and three orders of magnitude respectively." In the absence of an alternative explanation for the discrepancy between the measured and calculated $P_{50(7.4)}$, the increases in ADP observed with the hypoxic exposure suggest that a cause and effect relationship is plausible. More extensive experimentation would have to be completed to confirm such a relationship.

It would be logical to look for a mechanism which could, at least qualitatively, account for these ADP increases. On a purely speculative basis, the increase in glycolysis under alkalotic conditions, should be reflected by an increase in ATP, hence a decrease in ADP in the absence of increased ATP utilization and/or ADP generation. The increase in MCHC (decrease in cell volume) possibly partially accounts for the absence of such ATP increases due to energy utilization for the maintenance of cell size and shape. In the event that only some of this additional ATP is used for such maintenance, the balance could accelerate the reaction:



This reaction was proposed by Brewer (1969) as an alternative to oxidative phosphorylation for the indirect generation of ATP by the mammalian red cell through AMP; the ADP product thus generated would be converted to ATP via the glycolytic pathway. If increased glycolysis does not keep pace with increases in ADP, ADP would accumulate. Additional acceleration of the forward reaction would occur if AMP concentrations increased and/or the activity of adenylylase was enhanced to favor the forward reaction.

Brewer (1969), in his excellent review of ATP, points out that unlike the human red cell, the rabbit erythrocyte has the capacity to carry out the final steps of de novo adenine synthesis. Furthermore, the rabbit red cell has the capability of utilizing adenine, hypoxanthine, inosine, and adenosine as ATP precursors; such capacity is restricted to adenosine for the human red cell. An increase in any of these precursors in the rabbit could result in increases in adenosine phosphate.

Conclusion.

The results of this study show that exposure of male rabbits to an isobaric hypoxic (8.8% O₂) environment causes a significant increase in the plasma ESF concentration within 5 hrs exposure. Addition of 5.6% or 10% CO₂ to the hypoxic gas mixture almost completely blocks this response. Since the whole-blood XL accumulation observed with hypoxia alone is also blocked with the hypoxic-hypercapnic exposures, it appears likely that the ESF suppression is the result of enhanced oxygen delivery to the ESF producing tissue (s). This increase in tissue oxygenation probably occurs in response to the combined effect of increased pulmonary ventilation and blocking of the leftward P_{50(i.v.)} shift.

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