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Effects of Carbon Monoxide or Low Oxygen Gas Mixture Inhalation on Regional Oxygenation, Blood Flow, and Small Vessel Blood Content of the Rabbit Heart

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Abstract. The effects of lowering arterial O_2 content, approximately 30%, by inspiration of low O_2 or CO gas mixtures on regional myocardial relative tissue P_{O_2}, perfusion and small vessel blood content were studied in anesthetized, thoracotomized New Zealand white rabbits. Relative tissue P_{O_2} and perfusion were determined polarographically. $^{59}FeCl_3$ was used to determine small vessel blood content. In control, relative tissue P_{O_2}, perfusion and small vessel blood content averaged 33.1 mm Hg, 64.9 ml/min/100 g and 4.3 ml/100 g respectively in the subepicardium (EPI) and 22.7, 53.6 and 4.2 in the subendocardium (ENDO) of the left ventricle. Both hypoxic conditions increased regional blood flow, but to a lesser extent in the ENDO. Relative ENDO tissue P_{O_2} fell more markedly than EPI in both conditions. Small vessel blood content increased more with CO than low O_2. Regional O_2 consumption, calculated by Krogh analysis, increased under both conditions. The response to lowered O_2 content is thus an increase in flow, metabolic rate and the number of open capillaries with a lowered driving pressure for O_2. The effects of these types of hypoxia appear more severe in the ENDO.

Key words: Hypoxia – Carbon monoxide – Relative tissue P_{O_2} – Coronary perfusion – Open capillary density.

Introduction

When arterial oxygen content is lowered, due to inhalation of CO or low oxygen containing gas mixtures, there is an attempt throughout the body to maintain oxygen consumption at normal levels. The primary aim of the present study is to examine the response of the myocardium to a hypoxic stress in terms of its attempts to maintain O_2 delivery to the tissue. Both hypoxic-hypoxemia and CO-induced hypoxemia lead to a reduction in arterial O_2 content. Hypoxic-hypoxia stimulates arterial chemoreceptors, whereas CO-induced hypoxia, which leads to a leftward shift in the hemoglobin dissociation curve [4, 5], may not. One portion of the myocardial response is an increase in coronary blood flow with both stresses [1, 3, 5, 6, 18, 19, 30]. Further, there is evidence that hypoxic-hypoxemia causes a decrease in intercapillary distance, at least on the surface of the heart [7]. Myocardial tissue P_{O_2} also declines under severe hypoxia [21]. No measurements of the response of capillaries or tissue P_{O_2} to the effect of carbon monoxide in the heart are available. However, brain and muscle tissue P_{O_2} has been shown to drop in response to inhalation of low levels of CO [23].

Under many stressful conditions, it appears that the subendocardial (ENDO) region of the left ventricular free wall is more vulnerable than the more superficial subepicardial (EPI) region [13, 22]. In hypoxic-hypoxemia EPI perfusion appears to increase to a proportionally greater extent than ENDO [8]. Myocardial tissue P_{O_2} has been demonstrated to decline in hypoxia [21], but regional observations have not been adequately pursued. No studies of CO-induced hypoxia on regional myocardial parameters have been performed to our knowledge. Therefore, we designed the present project to study the effects of reducing O_2 content employing two types of hypoxic stresses on a regional basis within the left ventricle, examining the relationship between myocardial oxygen supply and demand.

In order to examine and compare the differential myocardial effects of hypoxic-hypoxia versus CO-induced hypoxia, measurements of tissue perfusion, relative tissue P_{O_2}, and small vessel blood content, used as an indirect indication of open capillary density in the

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subepicardium and subendocardium were conducted. On the basis of the data obtained, analysis was performed to estimate metabolism for both regions under control and experimental conditions using the Krogh-Erlang equation [12]. Exposure to both 8% O_2 and 1% CO in 40% O_2 was selected to achieve an approximate 30% reduction in the arterial oxygen saturation, thus, establishing a similar degree of hypoxic stress to the myocardial oxygen supply.

Methods

Fifty New Zealand white rabbits of both sexes, weighing between 1.2 and 2.3 kg were employed in this study, which consisted of three separate series of experiments. The surgical protocol was uniform with regard to all three experimental series. Sodium pentobarbital was administered i.v. via the circumflex ear vein in a dosage of 30 mg/kg. This dosage was supplemented as required by the individual animal.

Operative Procedure. The femoral artery was isolated and cannulated with polyethylene tubing. This cannulation made possible the measurement of arterial blood pressure and heart rate. In addition, the catheter served as a route for the administration of supplemental anesthesia, as well as for the withdrawal of blood samples.

An endotracheal tube was inserted into the trachea. The tube was attached to a Harvard respirator, which served to provide positive pressure artificial respiration with room air and other gas mixtures. A left thoracotomy was performed at the fifth costal interspace, followed by a partial pericardotomy. Eucapnia was maintained by adjustment of the respirator throughout the entire period of experimentation, and monitored by a Godart-Statham Capnograph. Relative tissue P_{O_2} and H_2 clearance electrode outputs, along with heart rate and blood pressure readings were recorded with a Beckman Dynograph Recorder – R411.

Regional Blood Flow Determination: Hydrogen Clearance Measurements. Tissue perfusion of the left ventricle using the hydrogen clearance method was determined in 18 rabbits. Two bare-tipped platinum electrodes, 177 μ in diameter, were inserted into the myocardium, one in the subepicardium and the other in the subendocardium at depths of 1.5 mm and 3 mm respectively, under the surface of the left ventricle. A reference electrode of silver-silver chloride placed under the skin was connected to an electrometer, as were the bare-tipped platinum electrodes. The wire material for the prepared electrodes was composed of 90% platinum and 10% iridium insulated by a Teflon coating of which approximately 0.2–0.3 mm was removed from the tip. The rabbits were respired with a mixture of 2% H_2 gas in air until a steady state, depicted by a plateau in the deflection, was noted. The H_2 gas mixture was replaced by room air, and a desaturation curve was recorded. Blood flow was calculated from the $T_{1/2}$ of the semi-logarithmic plot of the H_2 clearance. Regional tissue perfusion values in ml/min/100 g were obtained using the first order equation $Q = \frac{\lambda 1n2}{T_{1/2}}$, where λ = blood/tissue perfusion coefficient considered to be 1.00 [2] and $T_{1/2}$ was the half time in minutes. The mathematical considerations of local blood flow were based on the theoretical contribution of Kety. This method has been described extensively [9, 22, 25, 27].

A washout curve representing a control was obtained. The animals were then allowed to breathe 2% H_2 gas for 10 min followed by 2% H_2 in 8% O_2 for 5 min, after which 8% O_2 gas inhalation was administered. A washout curve was obtained. After desaturation was established, the animal was allowed to breathe room air for 20–30 min. The H_2 clearance procedure was conducted, serving as a new control. The effect of a 3 min exposure of 1% CO in 40% O_2 followed by 10 min of room air, on blood flow was determined by a subsequent washout. Finally, the animal was exposed to the CO gas mixture for 3 additional minutes, followed by 10 min of room air breathing. Blood flow under this condition was also determined by H_2 clearance. After each washout curve, blood pressure and heart rate were monitored and anaerobic blood samples were collected.

Regional Relative Tissue P_{O_2} Determination. Polarographic determination of relative myocardial tissue P_{O_2} was conducted in 12 rabbits. The electrodes used in tissue P_{O_2} analyses were identical to those used for the blood flow determination. The electrodes were accepted as suitable for P_{O_2} recordings only when a linear response was demonstrated during calibration in 0.9% NaCl solution equilibrated with analyzed O_2 gas mixtures. Those electrodes selected for experimental use were calibrated before and after each particular experiment. The possibility of protein coating of the electrode tip while in the tissue and the subsequent washing off of the coating with recalibration preclude an absolute determination of tissue P_{O_2}. The methodological limitations have been discussed previously [22, 25, 27, 29].

Relative tissue P_{O_2} was recorded continuously during the course of the experiment. Control readings were recorded during steady state. Hypoxic-hypoxia was induced by respiring the animals for at least 10 min with 8% O_2, until a relative steady state was achieved. Room air was then given for 20–30 min. The animal was then ventilated with 1% CO in 40% O_2 for 3 min followed by 10 min of room air breathing, after which a recording was obtained. The entire last step was repeated, so that in total, CO inhalation lasted 6 min. In each section of the protocol blood pressure and heart rate were recorded and anaerobic blood samples collected for analysis. Blood samples were analyzed for arterial P_{O_2}, and P_{CO_2} employing the Instrumentation Lab Inc. Blood Gas Analyzer-313. O_2 saturation and %COHb were determined with a CO oximeter.

Small Vessel Blood Content. The rabbits were assigned to four groups containing five animals in each group. The experimental procedures of the various groups were conducted in a random order to avoid bias. The first group was a control group. The second group represented the hypoxic-hypoxia group which received a 6 min exposure to 8% O_2. The third group breathed 1% CO in 40% O_2 for 3 min, followed by 10 min of room air breathing and this procedure was repeated so that the total CO exposure was 6 min. Lastly, one group was asphyxiated by shutting off the respirator for 5 min. Blood samples for subsequent blood gas analyses, and heart rate and blood pressure were taken before and after each experimental condition.

The labelling of plasma siderophilin with $^{59}FeCl_3$ was used to measure the small vessel blood content. A dose of 50 μCi was administered and flushed with 0.5 ml of saline through the femoral catheter. Siderophilin ^{59}Fe is considered a more dependable assay [31] than labeled iron human serum albumin, since it remains in the circulation for a longer period of time. Two minutes post-injection of the radioisotope, two blood samples were collected. Details of this technique have been described previously [24, 26].

The heart was excised and submerged into a breaker containing an ice slurry, to be subsequently sectioned into the right ventricle, right septum, left septum, left ventricular subepicardium and subendocardium. Each section was individually rinsed in water and blotted 3 times to remove blood from large vessels, and placed in tared counting vials. The wet weight of the tissue and blood samples was obtained. The radioactive samples were counted in a Hewlett-Packard Automatic Gamma Counter. The small vessel blood content of the tissue samples was computed by dividing the radioactivity of the sample, less background, in cpm/mg, by radioactivity of the arterial blood samples in cpm/ml, less background.

Table 1. Blood pressure, heart rate and blood gas analyses

	Heart rate (beats/min	B.P. (mm Hg)	P_{O_2} (mm Hg)	P_{CO_2} (mm Hg)	Sa_{O_2} (%)	Sa_{CO} (%)
Control (1)	256.5 ± 8.4	92 ± 2.7	92.2 ± 2.5	34.3 ± 1.1	92.9 ± 2.0	
8% O_2	246.1 ± 9.3*	83 ± 2.7*	45.9 ± 3.6*	33.0 ± 1.1	70.2 ± 3.2*	
Control (2)	256.7 ± 7.4	85 ± 2.5	93.4 ± 2.5	36.2 ± 1.1	93.1 ± 1.3	1.0 ± 0.4
CO (1)	254.6 ± 8.8	77 ± 2.6*	91.1 ± 2.7	37.1 ± 1.5	76.2 ± 1.9*	21.0 ± 1.3*
CO (2)	238.5 ± 6.6*	75 ± 2.6*	90.0 ± 2.5	37.1 ± 1.6	71.2 ± 1.6*	28.0 ± 1.2*

* Significant at $P < 0.05$
Values are presented as mean ± S.E.
Under all circumstances, comparisons were made between control (1) and 8% O_2; and control (2) and CO (1 and 2)

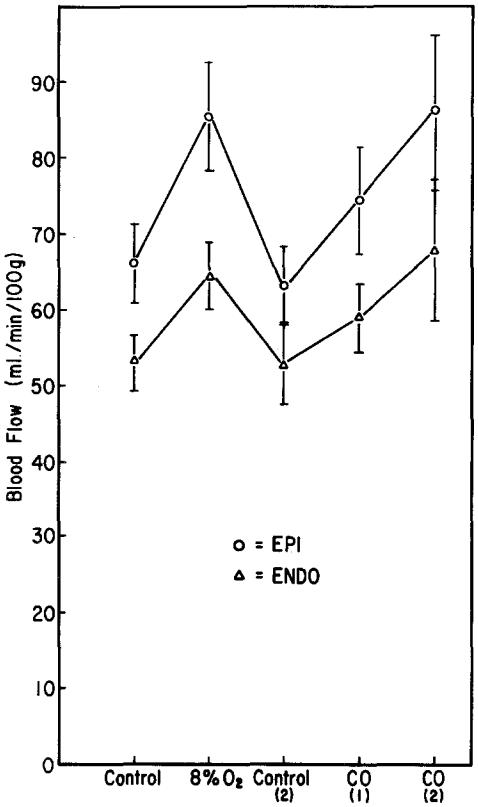

Fig. 1. Blood flow in ml/min/100 g tissue determined by H_2 clearance under various conditions is shown for subepicardium and subendocardium. Values were derived from recordings taken during relative steady state, and expressed as mean ± S.E.

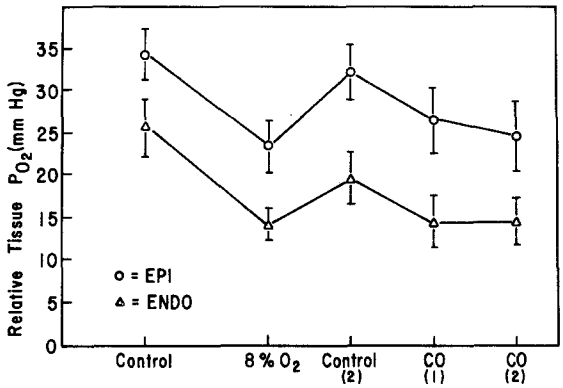

Fig. 2. Relative tissue P_{O_2} in mm Hg under control and experimental conditions is shown for subepicardium and subendocardium. Values were taken from recordings taken during relative steady state and expressed as mean ± S.E.

A variety of statistical analyses were performed on the data obtained. Analysis of variance-factorial ANOVA and Student-Newman-Keuls' test were employed in analysis of the small vessel blood content data. The effects of 8% O_2 and CO on blood pressure, heart rate, relative tissue P_{O_2}, and blood flow were analyzed by the Student *t*-test for paired means. Values used in analysis for each parameter were recorded during steady state. A value of $P < 0.05$ was accepted as significant in this study.

Results

The control values for blood pressure, heart rate and blood gas analysis are given in Table 1. Under control conditions, EPI tissue perfusion averaged for both control 1 and control 2, 64.9 ml/min/100 g and ENDO perfusion was 53.9 ml/min/100 g as measured by hydrogen clearance, Fig. 1. EPI relative tissue P_{O_2}, averaged for control 1 and 2, 33.1 mm Hg was significantly higher than ENDO, 22.7 mm Hg, Fig. 2. No regional differences were found in small vessel blood content under any of the tested conditions in the heart, Fig. 3. In control, small vessel blood content averaged 4.2 ml blood/100 g tissue. No significant differences were observed between the initial and second control period in any of the measured parameters. It should also be noted that the experiments were conducted maintaining eucapnia throughout; arterial P_{CO_2} was not significantly different under either experimental conditions, Table 1. A value of 1.0 ± 0.4% for Sa_{CO} was found in the control period.

Effect of 8% O_2 Inhalation

During the 10 min exposure to 8% O_2, there was a slight but statistically significant reduction in both arterial blood pressure and heart rate, Table 1. Arterial P_{O_2} fell 50.2% below the control value. Exposure to the

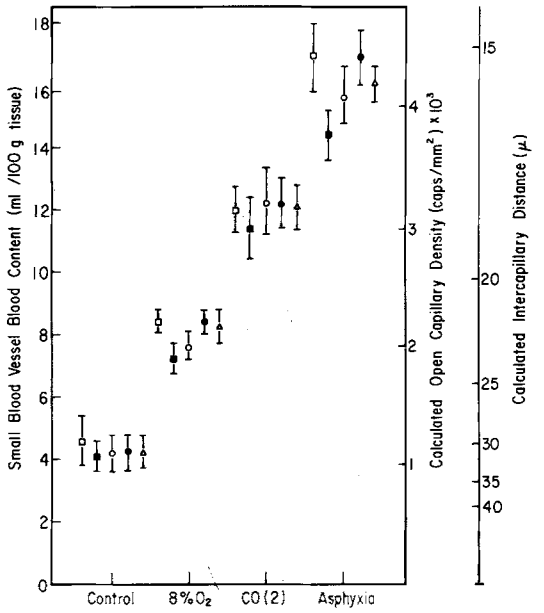

Fig. 3. Mean ± S.E. small vessel blood content in ml blood/100 g tissue under various conditions is shown for right ventricle (RV, □) right septum (RS, ■), left septum (LS, ○), left ventricular subepicardium (EPI, ●) and left ventricular subendocardium (ENDO, △). The first right ordinate represents calculated capillary density assuming a capillary radius of 2.5 μ and that 50% of the small vessel blood content resides in capillaries. The second right ordinate represents the corresponding calculated intercapillary distance

Table 2. EPI/ENDO ratio — regional comparison of tissue perfusion and relative tissue P_{O_2}

	Blood flow	Relative tissue P_{O_2}
Control (1)	1.13 ± 0.06	1.39 ± 0.11
8% O_2	1.24 ± 0.05*	1.69 ± 0.12*
Control (2)	1.13 ± 0.04	1.52 ± 0.08
CO (1)	1.21 ± 0.06	2.03 ± 0.18*
CO (2)	1.31 ± 0.09*	2.04 ± 0.21*

* Significant at $P < 0.05$
Values are presented as mean ± S.E.
Under all circumstances, comparisons were made between control (1) and 8% O_2; and control (2) and CO (1 and 2)

gas mixture resulted in a decrease in Sa_{O_2} to 70.2%, Table 1.

Figure 1 shows the effect of inhalation of 8% O_2 on regional myocardial blood flow. Tissue perfusion was increased significantly in both regions. The EPI region, however, showed a proportionally greater increase in flow as evidenced by the increase in the EPI/ENDO flow ratio, Table 2. A significant reduction in relative tissue P_{O_2} was evident in both the EPI and ENDO regions of the left ventricular free wall after exposure to the 8% O_2 gas mixture, Fig. 2. The significant increase in the EPI/ENDO P_{O_2} ratio, Table 2, illustrated the greater proportional decline in ENDO P_{O_2}. Figure 3 shows the results of exposure to the gas mixture on small vessel blood content. No regional differences were found. There was, however, a statistically significant increase in small vessel blood content over control after inspiring the 8% O_2 gas mixture to 8.0 ml blood/100 g tissue.

Effect of CO Inhalation

After a 3 min exposure to 1% CO in 40% O_2, an increase in carboxyhemoglobin saturation to 21% was noted along with a fall in O_2 saturation, Table 1. The partial pressures of O_2 and CO_2 were similar to control, 5 min after this treatment. Heart rate was not affected by this treatment, but blood pressure fell significantly. Myocardial tissue perfusion was elevated in both regions, but not significantly, Fig. 1. Relative tissue P_{O_2} decreased in both the EPI and ENDO regions after this 3 min exposure to 1% CO, Fig. 2. There was a proportionally greater fall in tissue P_{O_2} in the ENDO region as seen by the significant increase in the EPI/ENDO relative tissue P_{O_2} ratio, Table 2.

A double exposure to CO for two periods of 3 min each did not significantly affect arterial P_{O_2} or P_{CO_2}, Table 1. The O_2 saturation of the arterial blood fell to 71.2% and the carboxyhemoglobin level rose to 28%. The Sa_{O_2} 5 min after this second exposure to CO was not different than that during the exposure to the 8% O_2 gas mixture. Bradycardia and reduction in mean arterial blood pressure occurred after this exposure to CO.

Blood flow to the EPI and ENDO regions of the left ventricle was significantly above the control values as delineated in Fig. 1. The second exposure of CO increased flow proportionally more than the first exposure. The EPI/ENDO flow ratio increased significantly, indicating a greater proportional increase in EPI perfusion with this double 3 min exposure to CO. Figure 2 depicts that both EPI and ENDO relative tissue P_{O_2} were significantly lower than the control values, by 23% and 27% respectively. Thus, the ENDO relative tissue P_{O_2} fell to a greater extent than the EPI relative tissue P_{O_2}, Table 2. There were no regional differences in small vessel blood content under this condition. CO inhalation caused a rise in small vessel blood content to a mean value of 12.0 ml blood/100 g tissue. Small vessel blood content after CO exposure was significantly higher than both the control condition and the 8% O_2 exposure value.

Effect of Asphyxia

A group of 5 animals was asphyxiated in order to determine whether maximum small vessel blood content had been achieved during either of the above

hypoxic conditions in the rabbit myocardium. During the period of asphyxia, bradycardia as represented by a mean heart rate of 200.0 ± 2.5 (mean ± S.E.) were observed along with a decrease in blood pressure to a mean value of 62 ± 2 mm Hg. No regional differences in small vessel blood content were observed after asphyxia. The mean small vessel blood content was 16.0 ml blood/100 g tissue under this condition. Asphyxia elevated small vessel blood content 74%, 50% and 25% above control, 8% O_2 and CO values respectively. All of these differences were statistically significant. Therefore, the present study demonstrated that small vessel blood content was increased significantly in the following way: control < 8% O_2 < CO (6 min) < asphyxia.

Discussion

One of the primary responses of the myocardium to the approximately 30% reduction in Sa_{O_2} with low O_2 or CO treatment in attempting to maintain oxygen delivery to the tissue was an increase in coronary blood flow. Flow increases ranged regionally between 21% and 36%. This flow increase tends to preserve O_2 supply to the heart. These results corroborate other studies which find decreases in coronary resistance with hypoxia induced by either low O_2 or CO [6, 18, 19, 30] and increases in total myocardial blood flow under these stresses [1, 3, 5, 6, 18, 19, 30].

Coronary blood flow increased in both the EPI and ENDO regions of the left ventricle with either the stress of hypoxic-hypoxemia or CO-induced hypoxemia. Flow in the EPI region increased to a greater extent in both stresses as can be seen by the changes in the EPI/ENDO flow ratios, Table 2. Regional myocardial blood flow studies were performed under hypoxia by Flohr and Breull (1975). Employing radioactive microspheres, they found ENDO flow higher than EPI under control conditions. Moreover, they noted that although total flow increased with hypoxic-hypoxia, ENDO flow remained higher. Recalculation of their data, however, for the left ventricular free wall revealed that the EPI/ENDO flow ratio changed from 0.57 in control to 0.73 in hypoxia. This indicated a greater proportional increase in EPI flow, similar to our findings, Table 2. Thus the EPI region of the left ventricle appears better able to maintain its O_2 supply in the face of a hypoxic stress than the ENDO. There are no regional measurements of coronary blood flow with carbon monoxide.

Using the hydrogen clearance method of measurement of coronary blood flow, EPI flow tends to be higher than ENDO in control. Using an indicator uptake method, like microspheres, ENDO flow tends to be slightly higher [9]. These differences have been reviewed [13, 22]. Both methods, however, give similar results in stress or drug studies with regard to alterations in flow [9, 13, 22]. The limitations of the hydrogen clearance method for measurement of coronary blood flow has been discussed in detail previously [9, 28].

Another response of the myocardium to the hypoxic stresses imposed is the opening of capillaries and the reduction in intercapillary distance. We have used the blood content of small myocardial vessels as a measure of changes in intercapillary distance. Both hypoxic stresses significantly increased small vessel blood content. The values corresponding to the period of CO inhalation were not only significantly greater than control, but also significantly greater than the 8% O_2 values.

The limitations of our method of measurement of small vessel blood content have been discussed in detail previously [24, 26]. Measurements of small vessel blood content require that the label be uniformly distributed in the blood, that the label remain in the blood, that no differences in hematocrit between large and small vessels exist and that blood only remains in small vessels after treatment. A previous study has established no differences in arterial and venous specific activity after 90 s [26]. Moreover, a recent study in rabbit has demonstrated that the level of radioactivity remains constant for up to 15 min, of which the last five represent a period of asphyxiation [24]. In addition, it has been verified that the hematocrit of small cardiac vessels is the same as that in larger vessels [15]. Our treatment in heart should only leave blood in vessels below 100 μ in diameter [17].

On the basis of the figures obtained for the small vessel blood content, estimates of the number of open capillaries can be calculated, provided assumptions as to the radius of capillaries and the percentage of small vessel blood content in capillaries are made [15, 26]. A radius of 2.5 μ was assumed, as this value corresponded closely to estimates of reported radii for the rat heart [7]. In addition, we made the assumption that 50% of our measured small vessel blood content was in capillaries [15]. This assumes equal changes in all small vascular elements [26]. Our calculation of open capillary density would be in error if one vascular element changed more than another, e.g. venules more than capillaries. We then calculated open capillary density and intercapillary distance (IC) as:

$$\text{capillaries/mm}^2 = 1/2 \text{ (ml of blood/g of tissues)}/\pi (2.5 \times 10^{-3})^2$$

$$\text{IC}(\mu) = 1{,}000/\sqrt{\text{capillary density}} .$$

The mean open capillary density values for the control, 8% O_2, CO and asphyxia conditions were estimated to be 1,07 caps./mm^2, 2,036 caps./mm^2, 3,044 caps./mm^2 and 4,060 caps./mm^2, respectively, Fig. 3. These calculated values are closely in accord with the results of similar studies [7, 15, 26], in which the recruitment of capillaries and consequently a decrease in the intercapillary distance has been demonstrated under conditions where arterial P_{O_2} was reduced [7]. While oxygen supply (flow × arterial O_2 content) to the myocardium was similar for both hypoxic-hypoxia and CO-induced hypoxia, small vessel blood content was higher and calculated intercapillary distance lower with CO-induced hypoxia. Similar results were observed in rabbit brain [24]. Neither hypoxic-hypoxia nor CO-induced hypoxia fully open the heart's capillary bed. Adjustments made at the level of the capillary may be due to a lower capillary P_{O_2} and/or higher O_2 consumption. It is well known that CO shifts the hemoglobin dissociation curve to the left [4, 5]. Thus, although both hypoxic stresses result in the same arterial O_2 content and saturation, a lower end capillary P_{O_2} could be expected in the case of CO-induced hypoxia despite its higher initial arterial P_{O_2}. Unless O_2 extraction is widely different under the two forms of hypoxia, the leftward shift in the curve with CO would cause a lower capillary P_{O_2} at the same saturation. With respect to an alteration in metabolism it has been suggested that metabolic autoregulation is a factor governing open capillary density, in that smooth muscle adjusts small blood vessel patency in accordance with local tissue O_2 consumption.

The response of the myocardium to hypoxia is to increase blood flow and the number of open capillaries. The driving pressure for oxygen, another factor affecting oxygen delivery, however, declined in the present study. Relative tissue P_{O_2} declined to a comparable extent with both hypoxic and CO-induced hypoxia. Other investigators have also shown that with severe hypoxic-hypoxia, tissue P_{O_2} declines in the heart.

Both subepicardial and subendocardial relative tissue P_{O_2} declined with either type of hypoxic stress. With both types of hypoxia, ENDO relative tissue P_{O_2} declined to a proportionally greater extent than in the EPI region, Table 2. In the EPI region, O_2 supply was well maintained and intercapillary distance decreased, yet relative tissue P_{O_2} still fell. This could be due to an increase in the regional metabolic rate and/or a decrease in capillary P_{O_2} which reduces the P_{O_2} gradient. Similar arguments can be made for the ENDO region as well. In this case, however, the lack of maintenance of O_2 supply with hypoxia may be part of the reason for the greater fall in tissue P_{O_2} in this region.

Although the present polarographic method has been used extensively before [10, 22, 27–29], it should be recognized that the values obtained are not absolute, but rather provide only a relative measure of local tissue P_{O_2}. The shortcomings of measuring tissue P_{O_2} by our technique have been given a considerable degree of attention [11, 14, 20, 22, 28, 29]. Briefly, the primary limits are tissue damage and protein coating of the electrodes. The electrodes sense an area of larger than that damaged. Even though electrodes are calibrated before and after each experiment, a protein coating could have been washed off. This would bias our results on the low side. Relative values in a single site should not be affected by any of the methodological limitations. The present system is rapid, stable and provides good, high resolution traces, of a qualitative nature.

The response of the myocardium to hypoxia is thus an increase in blood flow and the number of open capillaries with a drop in tissue P_{O_2}. Using this information, it is possible to calculate an indirect measure which will give qualitative information on regional O_2 consumption.

If it is assumed that each capillary supplies a cylindrical section of the tissue enveloping it and that capillaries are arranged in parallel sheets, with no countercurrent flow, then the Kety modifications [12] of the Krogh-Erlang equations, for estimating tissue metabolism, may be employed. A 10% leftward shift in the Hb dissociation curve with CO and a capillary pH of 7.4 were assumed. The equations are as follows:

$$\bar{P}_o = P_o - A(\mathrm{mR}^2/4d)$$
$$c_o = c_a - (\mathrm{m}/2Q)$$

where $\bar{P}_o$ = mean tissue P_{O_2}; P_o = mean intracapillary P_{O_2}; m = oxygen consumption; d = diffusion coefficient for oxygen; R = half intercapillary distance; Q = blood flow; A = a function of R and capillary radius; c_o and c_a = mean capillary and arterial oxygen content respectively. This technique of estimating regional O_2 consumption indirectly has been used previously in heart, brain and skeletal muscle [10, 28].

It is clear that Krogh analysis can only be used to estimate qualitatively regional oxygen consumption, being dependent on the accuracy of the various measurements as well as the inherent inaccuracies of the Krogh analysis itself. The calculations nevertheless showed subendocardial O_2 consumption to be higher than subepicardial in the control state in the rabbit heart, 8.8×10^{-2} ml O_2/min/cm^3 and 7.1×10^{-2} ml O_2/min/cm^3, respectively. Oxygen consumption increased regionally under both stress conditions. However, it appears from our data that the EPI O_2 consumption increased to a proportionally greater extent than that of the ENDO. Calculated values indicated an increase in O_2 consumption by 27% with 8% O_2 and by 30% with CO-induced hypoxia in EPI, whereas ENDO values increased with 8% O_2 and CO,

24% and 19% respectively. It has also been shown by Krogh analysis that ENDO O_2 consumption was 20–30% higher than EPI O_2 consumption in the hearts of anesthetized open chest dogs by the same methodology [10]. Further evidence to support the findings of Krogh analysis are seen in quantitative determination of O_2 extraction by microspectrophotometry and flow by radioactive microspheres, which show ENDO O_2 consumption to be higher than EPI [25].

We calculated an increase in myocardial O_2 consumption with either form of hypoxia. This was despite a decrease in both mean arterial blood pressure and heart rate. Others have shown similar increases in oxygen consumption despite no change or a decrease in hemodynamic parameters [5, 16, 19]. The increase in myocardial O_2 consumption could be brought about by an increase in volume work. Cardiac output has been shown to increase under these forms of hypoxia [1, 3, 5]. It is also possible that contractility and shortening velocity may have been increased resulting in alterations in oxygen consumption [19].

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