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EFFECTS OF CHRONIC HYPERCAPNIA  
ON BLOOD DISTRIBUTION IN ORGANS

by

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SUBMARINE MEDICAL RESEARCH LABORATORY  
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## SUMMARY PAGE

### THE PROBLEM

Although the acute effects of exposure to increased CO<sub>2</sub> levels on blood distribution in organs are well documented, no systematic studies on the effects of chronic CO<sub>2</sub> exposure on blood distribution have been reported.

### FINDINGS

Using radioisotope methods, red cell volume (chromium-51) and plasma volume (iodine-125) were simultaneously measured in brain, liver, muscle and skin and their corresponding hematocrits determined in guinea pigs exposed for various periods up to 15% CO<sub>2</sub>. The brain blood volume was greatly elevated throughout the exposure period (30%), indicating a correlation to CO<sub>2</sub> tension rather than to pH.

The blood content of the liver and muscle decreased after one-day exposure and increased again during the subsequent exposure period. The blood content of the skin rose 60% after one-hour exposure and fell below control levels after three days and seven days.

### APPLICATIONS

The findings are of importance for scientists and submarine medical officers concerned with the effects of chronic CO<sub>2</sub> exposure. They are necessary for a better understanding of CO<sub>2</sub> induced blood volume changes affecting heat regulation and provide a basis for studies of the effects of CO<sub>2</sub> retention on blood volume and heat regulation in diving operations.

### ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MF12.524.006-9028B - Time Concentration Exposure Limits to Carbon Dioxide. The present report is No. 4 on this work unit. The manuscript was approved for publication on 24 November 1969 and designated as SubMed ResLab Report Number 603.

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## ABSTRACT

Using radioisotope methods to determine, simultaneously, red cell volume (chromium-51) and plasma volume (iodine-125) the blood content of the brain, liver, muscle and skin, and their corresponding hematocrits were measured in guinea pigs during chronic hypercapnia induced by prolonged exposure to 15% CO<sub>2</sub>. The respiratory acidosis produced under these conditions resulted in biphasic pH changes, uncompensated phase up to two days and compensated phase from three days on. During the uncompensated respiratory acidosis, the arterial P<sub>CO<sub>2</sub></sub>, after one hour exposure, rose from 45 to 123 mm Hg, and remained at the elevated level throughout the exposure period thus allowing analysis of the physiological changes due to Ph to be distinguished from those due to P<sub>CO<sub>2</sub></sub>. The blood content of the brain was greatly elevated throughout the exposure to CO<sub>2</sub>, indicating a correlation with P<sub>CO<sub>2</sub></sub> rather than pH. The blood content of liver and muscle decreased significantly after one day of exposure and increased again during the subsequent exposure period. The blood content of the skin rose 60% after one hour exposure and fell below control values at three and seven days of exposure. Changes in blood volume observed during chronic hypercapnia in liver, muscle and skin have proved to follow the time course of pH changes.

# EFFECTS OF CHRONIC HYPERCAPNIA ON BLOOD DISTRIBUTION IN ORGANS

## INTRODUCTION

Although a large number of studies have been carried out on the effects of acute exposure to elevated CO<sub>2</sub> on blood flow in different organs, little or no information is available on the effects of chronic hypercapnia on blood flow as well as blood distribution in specific organs.

During acute exposure to increased carbon dioxide levels, two factors known to affect organ blood flow and vascular resistance are present, these are a decreased blood pH and an increased P<sub>CO<sub>2</sub></sub>. It is obviously difficult to differentiate between the effects of these two agents under these conditions. However, in chronic hypercapnia the effects of these two factors become specific inasmuch as the pH in blood and tissues undergoes biphasic changes known as uncompensated and compensated respiratory acidosis while the CO<sub>2</sub> tension remains rather constant throughout the whole exposure period.

The studies reported in this paper were undertaken to determine the effects of chronic hypercapnia on the blood content of brain, liver, muscle and skin of guinea pigs and to demonstrate the relationships to pH or P<sub>CO<sub>2</sub></sub> changes.

## MATERIALS AND METHODS

Male guinea pigs of the Hartley strain, weighing between 350 and 600

grams, were obtained from a commercial source. The animals were daily checked for any disease or deformities by a resident veterinarian and only those which showed an increase in body weight and no observable signs of infection were used in experiments.

Animals were exposed to 15 percent CO<sub>2</sub> in air (21 percent oxygen) for varying lengths of time in a plexiglass chamber rigged with food and water facilities. The gases used were obtained commercially and analyzed with a Scholander apparatus. The CO<sub>2</sub> concentration in the chamber was continuously monitored with a Beckman infrared CO<sub>2</sub> analyzer and the oxygen concentration intermittently with a Beckman O<sub>2</sub> analyzer. With these instruments the CO<sub>2</sub> concentration was held at 15 ± 0.5 percent and the oxygen at 21 ± one percent. The chamber was installed in an air conditioned room which maintained the ambient chamber temperature at 24 ± 2° C. Air within the chamber was continuously circulated through silica gel containers by means of a closed-circuit system. Another closed-circuit system, within the chamber, was equipped with boric acid containers to remove ammonia vapor. These systems maintained the environmental humidity at 65-75 percent. The exposure chamber was opened daily for a short time (2-3 minutes) for the removal of urine and feces and the replenishment of food and water.

Due to external circumstances, the values of one day recovery on air were

obtained after six days of exposure to 15 percent CO<sub>2</sub> rather than after seven days as was originally planned.

Other studies in our laboratory have shown that the acid-base status of six days and seven days of exposure to 15 percent CO<sub>2</sub> is not significantly different.

The blood volumes of the various tissues were done by modification of methods described by Dewey<sup>10</sup>. The animals were injected intravenously via the jugular vein with reconstituted blood tagged with CR-51 and I-125 prepared as previously described, Baker and Schaefer<sup>2</sup>. Whole blood samples were taken after 15 minutes and assayed as previously described. All tissue samples were taken as soon as the blood sampling was completed. The animals were under the same light pentobarbital anesthesia (26 milligrams/Kg bodyweight) that was used in all other procedures. The skin was obtained from the abdominal region. Care was taken to remove all adhering muscle and adipose tissue. The skin tissue was then blotted with surgical gauze and placed in pre-weighed glass test tubes. Muscle tissue obtained was a composite of abdominal musculature and was treated as the skin sample. The liver was removed next. The descending aorta and the inferior vena cava were clamped with mosquito hemostats, after which the liver was quickly excised, blotted with surgical gauze and weighed minus the gall bladder. The animal was then decapitated and the whole brain, including the medulla oblongata, removed intact. Two samples of each tissue were taken (the brain being divided in half) and

weighed in pre-weighed test tubes with a Torbal Top Loading Balance to  $\pm 2$  mg. Tissue samples were digested for 24 hours with an equal volume by weight of KOH (38 percent) as described by Martin et al.<sup>20</sup>. The tissue samples were then counted three times for a minimum of 15 minutes of 10,000 counts with a Picker Spectrascaler II for CR-51 and I-125.

Calculations:

$$(a) \text{ Red cell wt. per gm of tissue} = \frac{\text{cpm Cr-51 per gm RBC (circulating)}}{\text{cpm Cr-51 per gm tissue}}$$

$$(b) \text{ Plasma wt. per gm of tissue} = \frac{\text{cpm I-125 per gm plasma (circulating)}}{\text{cpm I-125 per gm tissue}}$$

$$(c) \text{ Blood volume per gm of tissue} = (\text{Plasma volume per gram tissue} + \text{red cell volume per gm tissue}) \times \text{total weight of the tissue}$$

$$(d) \text{ Tissue hematocrit} = \frac{\text{red cell volume per gm tissue}}{\text{red cell} + \text{plasma volume per gm tissue}} \times 100$$

The weight of the muscle mass was taken to be 45 percent of the guinea pig body weight, Caster et al.<sup>8</sup>; Pace and Rathbun<sup>29</sup>; and Pace and Rathbun<sup>25</sup>. The skin mass was determined to be 19 percent of the total body weight, based on measurements of six guinea pigs.

The weight of one ml. of whole blood, as determined in duplicate on 12 guinea pigs to be 1.0271 + .004 grams, was

used to convert the weight values to volume. Since the net amount of blood removed by sampling was less than five percent of the initial blood volume no correction was made for this loss in determination of the tissue blood volumes, Gibson et al.<sup>13</sup>. All blood tissue sampling was done at the same time of day (between 11 AM and 12 noon) to avoid any possible errors due to circadian oscillations in the parameters studied.

## RESULTS

Data on brain red cell volume, plasma volume, total organ blood volume and hematocrit obtained during chronic hypercapnia are presented in Table I. Brain blood volume increased 30 percent after one hour of exposure to 15 percent carbon dioxide, remained at this elevated level throughout the seven day exposure and returned to initial values after one day recovery following six days exposure to 15 percent CO<sub>2</sub>.

The increase in brain blood volume was mainly accomplished through the rise in red cell volume while the plasma volume increased less. This is expressed in the persistent elevation of the brain hematocrit during CO<sub>2</sub> exposure which reached a maximum of 0.56 after three days of exposure compared with a control level of 0.36.

Liver blood volume (Table II) showed a decrease during the first three days of exposure which reached statistical significance after one day exposure and was followed by a return to control levels after seven days of exposure.

The fall in liver blood volume was based on a decrease in liver blood plasma volume; the red cell volume increased throughout the exposure to CO<sub>2</sub>. After one day recovery on air, following six days of exposure to 15 percent CO<sub>2</sub>, liver blood volume fell 50 percent below control values. This time both red cell volume and plasma volume decreased but the former dropped comparatively more, resulting in a decline of the liver hematocrit to initial values.

Muscle blood volume (Table III) exhibited biphasic changes similar to those observed in liver blood volume with the exception of a tendency to increase during the first hour of exposure. The greatest reduction in muscle blood content (-30%) which occurred after one day of exposure to 15 percent CO<sub>2</sub> was found to be related to a significant decrease of both red cell and plasma volume and a decrease in muscle hematocrit. The subsequent increase of muscle blood content to 20 percent above control level at seven days of exposure to 15 percent CO<sub>2</sub> was accompanied by an increase in hematocrit. During recovery from hypercapnia, muscle blood volume also fell sharply (-36%) and the hematocrit dropped to values markedly below control levels.

The skin blood content showed biphasic changes in chronic hypercapnia, but in opposite direction to those observed in muscle and liver blood content. There was a nearly 60 percent rise in skin blood volume after one hour of exposure to 15 percent CO<sub>2</sub>. At three and seven days exposure skin blood volume was found to decrease

Table I. Brain Red Cell and Plasma Volume/Total Blood Volume and Hematocrit in Guinea Pigs During Chronic Hypercapnia

Condition	N	Red Cell Volume $\mu\text{l/gm}$	Plasma Volume $\mu\text{l/gm}$	Blood Volume $\mu\text{l/gm}$	Total Volume Percent Changes from Control	Hct
Control	13	$5.76 \pm 2.63$	$9.99 \pm 4.04$	$15.83 \pm 5.42$	0	.36
<u>15% CO<sub>2</sub></u>						
1 Hour	5	$10.28 \pm 1.71^*$	$12.42 \pm 3.60$	$22.50 \pm 4.43^*$	+ 29.7	.457
6 Hours	4	$8.25 \pm 1.68^*$	$11.45 \pm 1.80$	$19.70 \pm 3.40^*$	+ 19.7	.41
1 Day	6	$8.90 \pm 2.27^*$	$13.98 \pm 3.01^*$	$22.88 \pm 4.71^*$	+ 30.8	.39
3 Days	8	$13.08 \pm 3.24^*$	$9.5 \pm .72$	$23.28 \pm 3.80^*$	+ 32.0	.57
7 Days	5	$11.80 \pm 1.78^*$	$13.35 \pm 3.94$	$25.15 \pm 2.78^*$	+ 37.0	.46
<u>Recovery</u>						
1 Day on air post 6 day exposure to 15% CO <sub>2</sub>	3	$4.03 \pm .697$	$10.50 \pm 2.30$	$14.56 \pm 2.30$	- 8.0	.27

Values are means plus S. D.

\*Statistically significantly different from control at the 5% level and better.

Table II. Liver Red Cell and Plasma Volume/Total Blood Volume and Hematocrit in Guinea Pigs During Chronic Hypercapnia

Condition	N	Red Cell Volume $\mu\text{l/gm}$	Plasma Volume $\mu\text{l/gm}$	Blood Volume $\mu\text{l/gm}$	Total Volume Percent Changes from Control	Hct
Control	13	28.52 $\pm$ 3.47	111.22 $\pm$ 10.31	139.30 $\pm$ 7.6		.26
<u>15% CO<sub>2</sub></u>						
1 Hour	5	33.18 $\pm$ 4.71	95.17 $\pm$ 21.9*	128.32 $\pm$ 36.2	-9	.35
1 Day	6	34.92 $\pm$ 5.52*	80.37 $\pm$ 21.5*	115.60 $\pm$ 28.9*	-17	.43
3 Days	8	39.45 $\pm$ 9.5*	92.05 $\pm$ 22.6*	131.5 $\pm$ 27.7	-6	.43
7 Days	5	38.09 $\pm$ 12.74	103.76 $\pm$ 20.99	141.85 $\pm$ 33.7	+ 1.8	.37
<u>Recovery</u>						
1 Day on air post 6 day exposure to 15% CO <sub>2</sub>	3	12.89 $\pm$ .40*	57.28 $\pm$ 21.98*	70.17 $\pm$ 22.3*	-50	.23

\* Statistically significantly different from control at the 5% level and better.

Table III. Muscle Red Cell and Plasma Volume/Total Blood Volume and Hematocrit in Guinea Pigs During Chronic Hypercapnia

Condition	N	Red Cell Volume $\mu\text{l/gm}$	Plasma Volume $\mu\text{l/gm}$	Blood Volume $\mu\text{l/gm}$	Total Volume Percent Change from Control	Hct
Control	13	$7.54 \pm 5.52$	$10.6 \pm 5.80$	$18.18 \pm 3.26$		.415
<u>15% CO<sub>2</sub></u>						
1 Hour	5	$7.53 \pm 2.08$	$11.35 \pm 3.65$	$18.88 \pm 3.82$	+4	.40
1 Day	6	$4.50 \pm 1.52^*$	$8.82 \pm 3.38^*$	$13.06 \pm 3.55^*$	-28	.344
3 Days	8	$6.51 \pm 3.20$	$11.43 \pm 4.60$	$17.94 \pm 2.71$	- 1	.362
7 Days	5	$9.03 \pm 5.2$	$11.61 \pm 6.02$	$21.64 \pm 6.3$	+19	.417
<u>Recovery</u>						
1 Day on air post 6 day exposure to 15% CO <sub>2</sub>	3	$3.43 \pm 1.46^*$	$8.26 \pm 1.85^*$	$11.68 \pm 3.2^*$	-36	.294

\* Statistically significantly different from control at the 5% level and better.

Table IV. Skin Red Cell and Plasma Volume/Total Blood Volume and Hematocrit in Guinea Pigs During Chronic Hypercapnia

Condition	N	Red Cell Volume $\mu\text{l/gm}$	Plasma Volume $\mu\text{l/gm}$	Blood Volume $\mu\text{l/gm}$	Total Volume Percent Changes from Control	Hct
Control	6	$5.27 \pm 1.57$	$8.96 \pm 1.81$	$14.25 \pm 4.17$		.37
1 Hour	5	$8.30 \pm 1.79^*$	$14.08 \pm 1.57^*$	$22.37 \pm 2.37^*$	+ 57%	.37
3 Days	4	$4.25 \pm .19$	$6.4 \pm 1.10^*$	$10.8 \pm 1.18$	- 16%	.39
7 Days	4	$3.39 \pm .92^*$	$5.10 \pm 1.62^*$	$8.50 \pm 2.09^*$	- 40%	.39

\* Statistically significantly different from control at the 5% level and better.

below control levels, reaching 40 percent after seven days. In spite of these large fluctuations in skin blood volume, the hematocrit remained practically constant.

In Figure 1, changes in pH and  $\text{PCO}_2$  measured during chronic hypercapnia are plotted together with the changes in organ blood content. The data show that the time course of  $\text{CO}_2$  tension appears to be correlated with that of brain blood content while the biphasic changes in the time course of pH

parallel the biphasic changes in blood content of muscle, liver and skin.

In this figure some additional data are shown which were obtained on a group of animals which did not exhibit any evidence of compensation of the  $\text{CO}_2$  induced respiratory acidosis after three days of exposure to 15 percent  $\text{CO}_2$ . This sign was found to signify that the animals would succumb within a matter of hours. Brain blood content measured in four animals belonging to such a group was found to be nine percent below control levels.

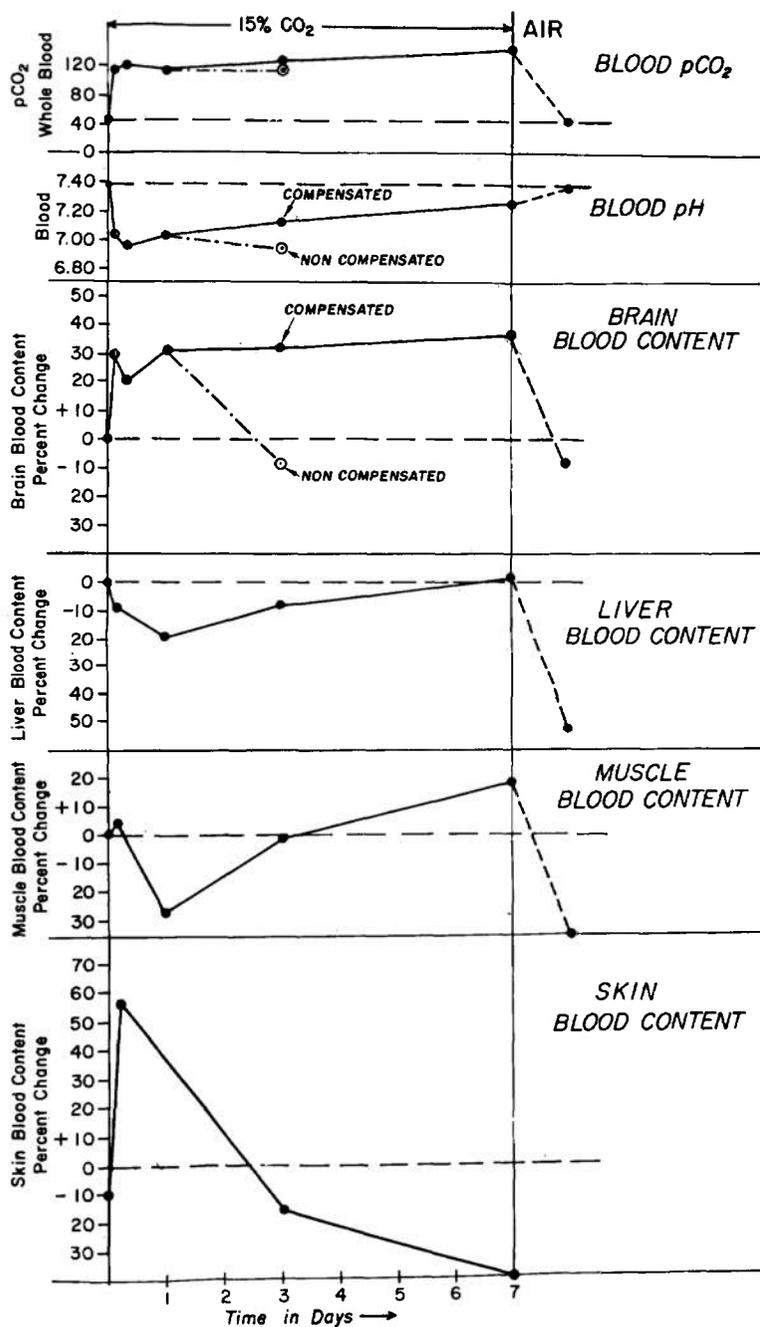


Figure 1. Blood  $P_{CO_2}$  and pH and percent changes of blood volume in brain, liver, muscle and skin during chronic exposure to 15%  $CO_2$ . Due to external circumstances the values of one day recovery on air were obtained after six days of exposure to 15%  $CO_2$  rather than after seven days as was originally planned. Since other studies in our laboratory have shown that the acid-base status of six days and seven days of exposure to 15%  $CO_2$  is not significantly different, dashed values denoting recovery period have been connected to the seven day exposure points.

A group of animals which failed to reach a partial compensation of the respiratory acidosis after three days of exposure to 15%  $CO_2$  are included for comparison of pH,  $P_{CO_2}$ , and brain blood content.

## DISCUSSION

The measurements of blood content of organs in chronic hypercapnia were carried out in pentobarbital anesthesia. Since the latter is known to affect both acid-base status and blood flow of the brain, the influence of anesthesia on the experimental results has to be evaluated.

Rather small effects of pentobarbital anesthesia on blood pH of small animals have been reported. Extracellular pH dropped 0.07 pH units and arterial CO<sub>2</sub> tension rose 4.7 mm Hg when measured prior to injection of barbiturate and after attainment of surgical anesthesia, Ponten and Siesjö<sup>27</sup>. The large alterations in pH (-.40 pH units), caused by prolonged exposure of guinea pigs to 15 percent CO<sub>2</sub>, could not be influenced significantly by the effect of anesthesia.

The effects of pentobarbital anesthesia on brain blood flow have been studied quite extensively but produced contradictory results, decreases and increases in blood flow were reported. These discrepancies have been resolved to a certain extent by Bienmueller and Betz<sup>6</sup> who demonstrated qualitative and quantitative differences in the effects of barbiturate on brain blood flow, depending on whether artificial or spontaneous respiration was used. In general, the former resulted in a decrease and the latter in an increase in blood flow. Pentobarbital anesthesia (5 mg/kg) caused, in cats during spontaneous respiration, an increase of 2 mm Hg in P<sub>CO<sub>2</sub></sub> and a fall of 0.02 pH units. These acid-base balance changes were associated with a decrease of cerebral

vascular resistance averaging six percent in a series of five animals<sup>6</sup>. If we assume that the decrease in vascular resistance is indicative of an increase in blood content, pentobarbital anesthesia induced by a low dose level could be responsible for a slight increase in blood content of the brain. This means that in an animal under pentobarbital anesthesia the cerebral blood vessels are more dilated than in an unanesthetized animal. The vasodilatory effect caused by CO<sub>2</sub> inhalation would therefore be less than in an unanesthetized animal. Kopperman<sup>18</sup> and Betz<sup>3</sup> provided evidence showing a reduction in the amplitude of cerebral vasodilation and blood flow increase during inhalation of the same CO<sub>2</sub> concentration in anesthetized animals as compared to unanesthetized animals.

In our experimental condition the sequence is reversed inasmuch as the CO<sub>2</sub> exposure occurs prior to the narcosis. Since 15 percent CO<sub>2</sub> has been shown to cause a maximal dilatation of cerebral blood vessels, Betz<sup>3</sup>, a maximal blood content is most likely present at the onset of anesthesia. It is, therefore, unlikely that anesthesia can cause any further dilatation of the cerebral blood vessels. Under the conditions of our experiments, the effects of anesthesia at a dose level of 26 mg/kg must be considered to be minimal.

Under heavy barbital anesthesia (75 mg/kg) Rieke and Everett<sup>33</sup> observed a reduction in blood content of brain of rats as compared with blood content determinations made in rats under light ether anesthesia<sup>12</sup>. At this dose level pentobarbital anesthesia most likely

has severe effects both on respiration and acid-base balance similar to those observed in our experiments in animals which were unable to achieve a compensation of the respiratory acidosis and in which the brain blood content fell nine percent below normal (Figure 1) in contrast to the compensated animals which showed a 32 percent increase in blood content of the brain at the same exposure time of three days.

It has been known for some time that elevated CO<sub>2</sub> in the blood exerts two competitive influences on circulation, a local direct vasodilator effect and a central sympathetic vasoconstrictor influence, Rein et al.<sup>31</sup>, Richardsen et al.<sup>32</sup>. Carbon dioxide acts, according to Rein<sup>30</sup>, similar to sympathetic stimulation and the different effects of carbon dioxide on various organs have been explained with differences in the threshold for sympathetic vasoconstrictor stimulation, e.g., brain and heart have a high threshold and manifest only vasodilator effects of CO<sub>2</sub>. The two antagonistic effects of carbon dioxide appear, therefore, to be part of a homeostatic circulatory regulation of carbon dioxide in which blood is shifted to vital organs (brain and heart) at the expense of the perfusion of organs such as liver and muscle.

The striking vasodilator effect of CO<sub>2</sub> on the brain, resulting in a large increase of cerebral blood flow, is well documented, Kety and Schmidt<sup>16</sup>, Patterson et al.<sup>26</sup>, Schieve and Wilson<sup>36</sup>, Betz<sup>3, 5</sup>.

This effect of CO<sub>2</sub> on brain circulation has been so clearly and reliably demonstrated that inhalation of ten

percent CO<sub>2</sub> has been used to test the response of instruments for measurement of brain blood flow, Abrams et al.<sup>1</sup>. The question whether the vasodilator effect is dependent on pH or CO<sub>2</sub> tension has not been unequivocally answered, some authors claim a better correlation to pH of the arterial blood, Kety<sup>16</sup>, Patterson<sup>26</sup>, Novak<sup>24</sup> while others found a better relationship to the CO<sub>2</sub> tension, Schieve and Wilson<sup>36</sup>. Betz and co-workers who measured the brain blood flow and simultaneously pH and CO<sub>2</sub> tension in blood and on surface of the brain found a closer correlation between local blood flow in the brain to the cortical pH than to the CO<sub>2</sub> tension, Betz<sup>5</sup>. Moreover, Betz had observed an adaptation of brain blood flow in cats who were repeatedly exposed for four hours daily to three or five percent CO<sub>2</sub>; the increase in brain blood flow found in the beginning of exposure declined subsequently, Betz<sup>5</sup>.

To our knowledge, no measurements of blood content in the brain during chronic hypercapnia have been reported. The increase in blood content of the brain, averaging about 30 percent, observed throughout the exposure to 15 percent CO<sub>2</sub> is in line with observations on brain blood flow and with measurements made previously on blood content of hypothalamus and cortex of guinea pigs exposed to 15 percent CO<sub>2</sub> using the method of Klein for blood content determinations, Schaefer<sup>34</sup>. The blood content of the brain was found to be increased about 30 percent and remained approximately at this elevated level throughout the seven-day exposure to 15 percent CO<sub>2</sub>.

These findings, demonstrating that the brain blood content does not follow

the biphasic changes in extracellular pH but remains elevated throughout the whole exposure period to CO<sub>2</sub> parallel with the time course of the CO<sub>2</sub> tension (Figure 1), indicate that the increase in brain blood content produced in chronic hypercapnia is dependent on CO<sub>2</sub> tension rather than on PH.

The liver blood content in chronic hypercapnia shows biphasic changes correlated with the time course of the extracellular pH, (Figure 1). The largest decrease of liver blood content was observed after one day exposure (17 percent) at the height of the uncompensated respiratory acidosis associated with maximal sympathetic stimulation, Schaefer, McCabe and Withers<sup>35</sup>. This finding is in line with the known effects of sympathetic stimulation on liver blood flow, resulting in a discharge of blood from the liver.

Moreover, Epstein et al.<sup>11</sup> found that a rise of the arterial P<sub>CO<sub>2</sub></sub> from 38 to 56 mm Hg, corresponding to inhalation of approximately 7.5 percent CO<sub>2</sub>, caused a 21 percent decrease splanchnic blood volume and a 15 percent decrease in hepatic blood flow in patients maintained on a respirator. In animal studies, hypercapnia was also found to cause an increase in splanchnic vascular resistance and a significant decrease in blood flow, Mohamed and Bean<sup>22</sup>, Ramlo and Brown<sup>28</sup>, Gollwitzer-Meyer<sup>14</sup>. The arterial and venous constrictive effects of increased CO<sub>2</sub> in blood perfusing splanchnic blood vessels were dependent on an intact splanchnic nerve supply (22, 28, 14). These studies deal only with acute effects of hypercapnia,

corresponding to the uncompensated phase of respiratory acidosis in our experiments, and the reported findings are in agreement with our results, with the exception of those of Takacs and Kalley (38) who observed a significant increase in liver blood flow in rats exposed to 20 percent CO<sub>2</sub> for four to six minutes. The difference in the effects of one hour exposure of guinea pigs to 15 percent CO<sub>2</sub> and exposure of rats to 20 percent CO<sub>2</sub> for four to six minutes is probably not related to differences in concentration and time of exposure but might better be explained with the heavy dose of pentobarbital used in the rat experiments (50 mg/kg) which most likely suppressed the central vasoconstrictor response to CO<sub>2</sub>. This notion is supported by observations showing that the striking vasoconstriction in the limbs caused by inhalation of 30 percent CO<sub>2</sub> for two minutes in conscious man was completely eliminated or changed to vasodilation under general anesthesia, McArdle and Roddie<sup>21</sup>.

During the compensated phase of respiratory acidosis after seven days of exposure, the blood content of the liver returned to initial levels (+ 2%). At this time the stress response and sympathetic stimulation had subsided, Schaefer, McCabe and Withers<sup>35</sup>.

The significant reduction in blood content of the muscle after one day exposure to 15 percent CO<sub>2</sub> and the large rise in skin blood content after one hour are in agreement with reports in the literature on blood flow changes of these two organs in acute hypercapnia. Betz<sup>3</sup> observed a vasoconstrictor effect on muscle blood flow in cats and dogs during inhalation of a

CO<sub>2</sub> mixture of one percent and above for a period of ten minutes. Muscle blood flow decreased nearly linearly when the CO<sub>2</sub> concentration was increased from one percent to nine percent CO<sub>2</sub> but little change occurred thereafter between nine percent CO<sub>2</sub> and fifteen percent CO<sub>2</sub>.

Betz also observed occasionally transient increases in muscle blood flow during the vasoconstrictor phase which lasted for one to three minutes and could bring the muscle blood flow temporarily back to the initial level. The possible explanation for these transient increases in muscle blood flow is a temporary dominance of the local vasodilator effect of CO<sub>2</sub> which might be an expression of oscillations occurring between local and central effects of CO<sub>2</sub>. This might also explain the observed slight but insignificant increase in muscle blood content we found after one hour of exposure to 15 percent CO<sub>2</sub>.

Simultaneously with the muscle blood flow, Betz<sup>3</sup> measured the skin blood flow during CO<sub>2</sub> inhalation, which was either not changed or increased. Similar findings of a vasoconstrictor effect in the muscles and a vasodilator effect in the skin during acute hypercapnia was reported for rats, Takacs<sup>38</sup>, and humans, McArdle and Roddie<sup>21</sup>.

Krough<sup>19</sup> observed in rabbits, given about 15 percent CO<sub>2</sub> to breathe which corresponded to a decline in the pH of about 0.4, a definite hyperemia of the ear.

Data reported in the literature on acute effects of carbon dioxide on skin blood flow are in line with our finding of a large increase of skin blood content after one hour exposure to 15 percent CO<sub>2</sub>.

All four organs studies in this experiment showed, during acute hypercapnia, changes in blood content which correspond with the changes in blood flow of these same organs (brain, liver, muscle and skin) in acute CO<sub>2</sub> exposure reported in the literature<sup>3, 11, 19, 21, 22, 26, 28</sup>.

Since, to our knowledge, no studies of blood flow in chronic hypercapnia have been reported in the literature, it is not possible to compare blood content and blood flow changes in chronic hypercapnia.

Transition to air following prolonged exposure to 15 percent CO<sub>2</sub> showed a marked difference in the response of brain as compared to those of liver and muscle. The latter showed a drastic fall in blood content way below initial levels while the brain blood content returned to near control values. This suggests that the CO<sub>2</sub> effects on blood volume regulation of the brain are more closely controlled than CO<sub>2</sub> effects on blood volume regulation of liver and muscle.

Similar observations have been made on CO<sub>2</sub> effects on blood flow regulation in different organs. The difference might be related to the absence of any significant degree of nervous control of cerebral circulation which makes

for the special homostatic role of CO<sub>2</sub> in the regulation of cerebral blood flow<sup>37</sup>.

Since red cell volume and plasma volume were measured simultaneously and independently of each other, the ratio of red cell volume/plasma volume in the tissues (tissue hematocrit) are reliable and can provide some additional information about the mechanisms of CO<sub>2</sub> action in different organs.

The brain hematocrit is greatly elevated throughout CO<sub>2</sub> exposure reaching an increase of 60 percent at three days exposure due to a large rise in red cell volume, while plasma volume does not rise significantly except at one day exposure. The discrepancy between the changes in red cell volume and plasma volume might be explained on the basis of a change in the permeability of the blood brain barrier caused by CO<sub>2</sub>.

Brierly<sup>7</sup> noted that inhalation of the 10-20 percent CO<sub>2</sub> caused an increase of the penetration of phosphate to the brain.

Clemenson et al.<sup>9</sup> have demonstrated that inhalation of 15 percent CO<sub>2</sub> for 15 minutes produces a permeability of brain capillaries in rabbits as indicated in trypan blue staining of cerebral tissue following dye injection. Although guinea pigs were not tested under 15 percent CO<sub>2</sub>, they were found to show the same sensitivity to CO<sub>2</sub> as rabbits<sup>9</sup>. Under those conditions, plasma will pass through the capillary walls and the brain plasma volume measured by I-125 would be less. Clemenson et al.<sup>9</sup> considered the possibility of carbon dioxide being a

physiological regulator of the permeability of the brain capillaries.

The transient decrease and subsequent increase in liver blood control is accomplished by changes in plasma volume, while the red cell volume remains at an elevated level throughout CO<sub>2</sub> exposure. As a consequence the liver hematocrit is consistently elevated during chronic hypercapnia.

Thickening of blood and filtering of plasma through the temporarily permeable walls has been described as one of the regular functions of the liver sinusoids<sup>17</sup>. They receive both venous and arterial blood from the portal vein and hepatic artery and drain the blood into the hepatic veins. With the help of outlet sphincters, blood can be retained in the sinusoids and plasma filtered out through the walls, resulting in a hemoconcentration. A CO<sub>2</sub> induced increase in permeability of the sinusoids could explain the elevated liver hematocrit. However, the biphasic changes in plasma content related to the uncompensated and compensated phase of respiratory acidosis require the operation of a pH dependent vasoconstrictor response.

The splanchnic venoconstriction produced by acute hypercapnia was found to be mediated through the Central Nervous System, the response disappeared when the splanchnic nerve supply was cut, Gollwitzer-Meier<sup>14</sup>, Mohammed and Bean<sup>22</sup>. This reflex pathway would be influenced by the increased sympathetic excitation associated with the first two days of exposure to 15 percent CO<sub>2</sub> which subsequently

subsides during the compensated phase of respiratory acidosis.

In spite of large variations of blood volume, the skin hematocrit remains amazingly constant. This suggests that a change in permeability of the capillary bed does not play a role as in brain and liver. The skin has an extensive system of arteriovenous anastomoses and the blood flow through these shunts has been shown not to participate in transvascular exchanges, Kety<sup>15</sup>. It is most likely that the large increase in skin blood content during acute hypercapnia and the subsequent decrease during chronic hypercapnia was accomplished by opening and closing of the arteriovenous shunts. The opening of the arteriovenous anastomoses has been found to be associated with excessive blood flow resulting in heat dissipation, Moreci et al.<sup>23</sup>.

The large increase in skin blood volume found during the acute phase of exposure to 15 percent could contribute significantly to the simultaneously observed fall in body temperature which was limited to the first two days of exposure to 15 percent CO<sub>2</sub>. \*

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13. ABSTRACT Using radioisotope methods to determine, simultaneously, red cell volume (chromium-51) and plasma volume (iodine-125) the blood content of the brain, liver, muscle and skin, and their corresponding hematocrits were measured in guinea pigs during chronic hypercapnia induced by prolonged exposure to 15% CO <sub>2</sub> . The respiratory acidosis produced under these conditions resulted in biphasic pH changes, uncompensated phase up to two days and compensated phase from three days on. During the uncompensated respiratory acidosis, the arterial P <sub>CO2</sub> , after one hour exposure, rose from 45 to 123 mm Hg and remained at the elevated level throughout the exposure period thus allowing analysis of the physiological changes due to pH to be distinguished from those due to P <sub>CO2</sub> . The blood content of the brain was greatly elevated throughout the exposure to CO <sub>2</sub> , indicating a correlation with P <sub>CO2</sub> rather than pH. The blood content of liver and muscle decreased significantly after one day of exposure and increased again during the subsequent exposure period. The blood content of the skin rose 60% after one hour exposure and fell below control values at three and seven days of exposure. Changes in blood volume observed during chronic hypercapnia in liver, muscle and skin have proved to follow the time course of pH changes.			

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