AD620287

NSAM-73

NAVAL SCHOOL OF AVIATION MEDICINE
U.S. NAVAL AIR TRAINING BASES
PENSACOLA, FLORIDA

RESEARCH REPORT

Submitted - 20 August 1945

PROJECT NO: X-409(Av-221-f)

REPORT NO: One

TITLE: THE RELATIONSHIPS IN VIVO BETWEEN CARBON MONOXIDE, OXYGEN AND HEMOGLOBIN IN THE BLOOD OF MAN AT ALTITUDE.

REPORT BY: Lieut J.L. Lilienthal, Jr., (MC) USNR

Lieut R. L. Riley, (MC) USNR

APPROVED: Captain Louis Iverson (MC) USN
Medical Officer in Charge

SUMMARY and CONCLUSIONS:

1. Three male subjects have been studied at varying pressure-altitudes while in equilibrium with inspired gas mixtures containing from 0.005 to 0.015 per cent carbon monoxide.

2. The distribution of COHb, O_2Hb and reduced Hb and their related gas tensions confirm in vivo the fundamental "laws" first defined by Haldane for the equilibria obtaining in vitro.

3. A simple rearrangement of the Haldane equation makes possible an accurate prediction of the amount of COHb obtaining when man is in equilibrium with a CO-contaminated atmosphere at any given altitude.

The equation,

$$(COHb) = \frac{MpCO}{pO_2 + MpCO} \times (\text{Total hemoglobin saturation})$$

requires only that the percentage of CO in inspired air and the pressure-altitude be known. The remainder of the terms may be read from standard values, tables and curves; e.g., $M = 210$, $pO_2 =$ average alveolar tension at the given pressure-altitude, and (Total hemoglobin saturation), related to the tension of $pO_2 + MpCO$, may be read off the standard oxyhemoglobin dissociation curve.

4. The value of the relative affinity constant of Hb for CO compared to O_2 was found to be 204 ± 10 p.c. in these experiments.

5. The total barometric pressure has been found to play no role in the distribution of CO and O_2 at equilibrium.

6. The hemoglobin of individuals who smoke appears not to differ from that of non-smokers in its affinity for CO and O_2.

7. The symptoms produced by CO are proportional not only to the blood concentration of COHb but also to the duration of exposure to a given concentration.

The limits for permissible contamination of inspired air by carbon monoxide have been set, according to current military and industrial specifications, to preclude any undesirable concentrations of carboxyhemoglobin when equilibrium has been reached. The initial rates of uptake of CO by man have been studied recently by several investigating groups (3, 7, 10). The cognate problem of the distribution of COHb, O_2Hb and reduced Hb and their related gas tensions has been studied in vitro with increasing precision from the original experiments of Douglas and the Haldanes to the most recent experiments of Roughton and Darling (2, 13). There is no information available, however, on the equilibria relations which obtain in vivo, especially at various pressure-altitudes. The study reported here was designed to furnish information on this phase of the general problem of CO intoxication.

METHODS:

Three male subjects were studied: CF, a light smoker; JL, a heavy smoker; and RR, a non-smoker. The subject's blood level was elevated abruptly toward an estimated equilibrium value at the beginning of each experiment by administering a mixture of 0.7 to 2.0 p.c. CO in air for 2 to 3 minutes (the "booster"). Then for periods ranging from 4 to 7 hours the subject lay at rest and breathed a mixture of CO, O_2 and N_2 through a close-fitting face mask from a demand regulator which metered the mixture delivered from a pressure tank.* With one exception the experiments were

* These gas mixtures were furnished through the kindness of the National Bureau of Standards. Owing to the fact that the mixtures of CO in air were compressed with re-cycle air in a liquid air compressor, the oxygen content was less than that of air. The oxygen content of each tank was determined by the Haldane technic and the pressure-altitude in the decompression chamber adjusted to give the desired inspired pO_2. The percentages of CO were stated by the National Bureau of Standards to be 0.005, 0.010 and 0.015. Analyses by a modification of the NBS method for CO agreed with the stated concentrations (4).

carried on at the desired pressure-altitude in a decompression chamber. At regular intervals blood was drawn from the antecubital vein and analysed for CO. When consecutive analyses indicated that equilibrium had been reached, an indwelling needle was introduced into the brachial artery and two samples of arterial blood were withdrawn at approximately 30 minute intervals. This experimental design insured that at the time of obtaining the arterial samples no further increment in (COHb) was to be expected.

The samples of venous blood were prevented from coagulating by the addition of dry potassium oxalate to produce a final concentration of 0.2 p.c. The samples of arterial blood were drawn directly into iced syringes containing 4 drops of liquid heparin with sodium fluoride. The following determinations were then carried out:

a) <u>pO_2</u> and <u>pCO_2</u> by the bubble method of Riley (12);

b) <u>CO contents by the Scholander-Roughton microgasometric method with</u> double quantities of blood (80 instead of 40 c.mm.). With this modification duplicate analyses checked within 0.05 vols p.c. in the 36 consecutive samples of blood analysed in this present study. The accuracy of the method is of this same high order;

c) <u>O_2 contents</u> by the Roughton-Scholander microgasometric method;

d) <u>total gas (CO) capacity</u> by the NIH photometer of Andrews and Horecker (1). The accuracy of this instrument for the estimation of the CO capacity of the blood has been established in this laboratory by comparative gasometric analyses, and the correspondence of the two methods is 0.2 vols p.c. or better;

e) <u>CO_2 contents of the whole arterial blood by the manometric method of</u> Van Slyke and Neill;

f) <u>pHs</u> of the arterial blood by a glass electrode of the MacInness and Belcher type (Cambridge Instrument Co.). The shielded assembly of the glass electrode, the internal silver-silver chloride electrode and the calomel reference electrode and all the solutions were maintained at a constant temperature of 37° C. in a warm air bath to eliminate all temperature gradient potentials. The glass electrode circuit was standardised with 0.05 M potassium acid phthalate and calibrated with Sørensen phosphate buffers. The pH of these solutions at 37° C. was kindly calculated for us by Dr. W. J. Hamer of the National Bureau of Standards.

RESULTS:

The hourly blood CO levels are presented in Figure 1. It will be noted that the blood concentrations of CO had reached constant levels at least one hour before the samples of arterial blood were drawn.

The pertinent data for each experiment are presented in Table 1.

DISCUSSION:

Haldane and his collaborators showed originally that the hemoglobin in a solution saturated with a mixture of CO and O_2 was distributed between COHb and O_2Hb according to the following expression (Haldane's first "law"):

$$\frac{(CO)}{(O_2)} = \frac{(COHb)}{(O_2Hb)} = \frac{MpCO}{pO_2} \qquad (A)$$

where M = the relative affinity constant of Hb for CO compared to O_2, and (CO) & (O_2) = gas contents of the blood in vols p.c., and (COHb) & (O_2Hb) = per cent saturation of the total hemoglobin (2).

Roughton and Darling have shown recently that the expression (A) holds true both for hemoglobin solutions and for whole blood even when an appreciable amount of reduced hemoglobin (Red. Hb) is present in the system (13). This demonstration in vitro also furnished experimental confirmation of Haldane's second "law": in blood exposed to O_2 at a partial pressure pO_2 and to CO at a partial pressure pCO the total hemoglobin saturation, $100 \times \frac{(COHb) + (O_2Hb)}{(COHb) + (O_2Hb) + (Red.Hb)}$, is the same as it would be in the absence of CO, if pO_2 then equaled $pO_2 + MpCO$. These relations imply likewise that the following pairs of functions may be described by the same standard oxyhemoglobin dissociation curve:

I) pO_2 & $100 \times \frac{(O_2Hb)}{(O_2Hb) + (Red.Hb)}$ in the absence of CO,

II) MpCO & $100 \times \frac{(COHb)}{(COHb) + (Red.Hb)}$ in the absence of O_2,

III) $pO_2 +$ MpCO & $100 \times \frac{(O_2Hb) + (COHb)}{(O_2Hb) + (COHb) + (Red.Hb)}$

The experimental data of this present study provide the means for putting Haldane's second "law" to the test in man. An inspection of Figure 2 shows that when the total effective gas tension is plotted against the total hemoglobin saturation (pair III above) the experimental points (solid circles) do in fact fall along the standard oxyhemoglobin dissociation curve. These findings confirm Haldane's second "law" when applied to equilibrium conditions existing in vivo.

Haldane's third "law" first drew attention to what is now termed the "Haldane effect": the presence of COHb shifts the oxygen dissociation curve of the remaining hemoglobin to the left (5). This "Haldane effect" has been re-examined and confirmed recently by Roughton and Darling in a series of precise experiments in vitro from which they have derived "some profitable simplifications in the development of the theory" (13). Their treatment of the data and the theory showed that the displacement of the oxyhemoglobin dissociation curve could be predicted through the use of the fundamental equation (A) and a standard oxyhemoglobin dissociation curve without knowledge of M or pCO or recourse to the Hill-Barcroft equation. Their assumption that "the effect of COHb on the O_2-dissociation curve in vivo should be quantitatively the same as the experimentally observed effect in vitro" has been confirmed in this present study: the open circles in Figure 2 (the in vivo dissociation of oxyhemoglobin in the presence of varying amounts of COHb) indicate by their displacement the "Haldane effect", and coincide with the calculated curves of Roughton and Darling (v. Figure 1 of their paper).

Although Roughton and Darling showed that a definition of the individual values of M and pCO were not needed to calculate the effect of COHb on the oxyhemoglobin dissociation curve, nevertheless, a knowledge of these factors adds to an understanding of equilibrium conditions in vivo. The data which are recorded in Table 1 afford an opportunity to calculate these factors.

<u>pCO</u> - The arterial pCO could not be determined directly. However, under the conditions of equilibrium existing in these experiments, the arterial pCO may be assumed to have been equal to the alveolar pCO, which could be calculated readily from the pCO of the inspired mixture (alveolar pCO = inspired pCO x (P_{bar} - 47)).

<u>M</u> - When the derived value for arterial pCO is substituted in a rearrangement of the fundamental equation (A) together with the other terms which have been determined directly, then

$$M = \frac{pO_2 \times (COHb)}{pCO \times (O_2Hb)} \qquad (A_1)$$

The validity of the assumption that arterial pCO = alveolar pCO gains support from the fact that the average value of M calculated on this basis (Table 1) is 204 ± 10 p.c. as compared with 210 ± 2.5 p.c determined in vitro by Sendroy, Liu and Van Slyke (14). This correspondence provides evidence that the same equilibrium is attained by the human subject as that which obtains in the tonometer.

The fundamental Haldane equation

$$\frac{MpCO}{pO_2} = \frac{(COHb)}{(O_2Hb)} \qquad (A)$$

can be rearranged after substituting

$$(O_2Hb) = (Tot.Satn) - (COHb) \qquad (B)$$

as follows:

$$(COHb) = \frac{MpCO}{pO_2 + MpCO} \times (Tot.Satn) \qquad (C).$$

Equation (C) is a useful expression which lends itself to the rapid estimation of the amount of COHb to be found in the blood of man in CO-equilibrium at any altitude through the use of generally accepted average values for M, pO_2 and (Tot.Satn). The use of equation (C) is demonstrated best by working through a sample calculation:

Given the problem of calculating the amount of COHb in the blood of a subject exposed to 0.008 p.c. CO in air at a pressure-altitude of 10,000 feet ($P_{bar} = 523$ mm.Hg) until equilibrium is reached, the following values would be substituted in equation (C)

$pCO = (523 - 47) \times 0.00008 = 0.038$ mm.Hg
$M = 210$ (Sendroy, Liu and Van Slyke, 14)
$pO_2 = 61$ mm.Hg (from Boothby's curve, 6)
(Tot.Satn) = 92 p.c. (read off a standard oxyhemoglobin dissociation curve (11) at a tension of $pO_2 + MpCO = 69$ mm.Hg).

whereby (COHb) = 10.7 p.c.

This calculation involves assumptions and values which have been tested only at rest and during mild exercise.

Evidence regarding the accuracy of this simplified method of estimating the equilibrium value of COHb at altitude is furnished by comparing values so calculated with those determined in our experiments (Table 1):

Per cent saturation COHb

Expt No.	Determined	Calculated	p.c.COHb
CF_1	7.5	7.0	+0.5
CF_2	7.2	7.2	0.0
JL_1	15.2	16.4	−1.2
JL_2	23.6	23.2	+0.4
RR_1	14.2	15.1	−0.9
RR_2	8.6	7.0	+1.6

The average deviation of the calculated from the determined value is less than 0.8 p.c.

Since the basic Haldane equation (A) describes the experimental data at all pressure-altitudes studied, it appears that variations in total barometric pressure do not affect equilibrium relations. This finding is consonant with the related observations that the uptake of CO by man at altitude is a function of pCO and pO_2 but not of P_{bar} (3, 7, 10).

There is no evidence in the data presented here to indicate that the presence of appreciable amounts of COHb in the blood of smokers changes the affinity of hemoglobin for CO or O_2. For example, the blood of subject RR was found to contain 0.2 vols p.c. or less of CO whenever examined in basal state; on the other hand, the blood of subject JL had a constant CO content of 1.2 to 1.5 vols p.c. Nevertheless, the affinities of these two individual hemoglobins for CO and O_2 were found to be virtually identical.

An estimate of the effect of a given concentration of COHb on certain physiological functions in man at altitude has been made difficult by the discordant results and conclusions reported by the several investigators who have examined this problem (8, 9, 15). The data recorded in this study complicate the practical problem further by indicating that the duration of exposure to a given concentration of COHb is a factor which cannot be ignored. For example, in two of the experiments in this series there were noted significant symptoms which appeared only after considerable time had elapsed. In experiment RR_1, the subject noted steadily increasing headache and recurrent nausea during the final 3 to 4 hours of exposure; in experiment JL_1, no symptoms were noted during the first hour, but thereafter there appeared headache which became progressively more severe, increasing and almost constant nausea, mental confusion, restlessness, pallor, cold extremities and a state of mild shock. These symptoms increased in severity as time passed although there was little if any change in (COHb), (O_2Hb) or the blood gas tensions during the course of the experiment. Thus, the symptoms produced by CO were related not only to the concentration of COHb but also to the duration of exposure.

The skillful technical assistance of D. D. Proemmel, PhM 1/c, WR, USNR, and R. E. Franke, S 1/c, WR, USNR, is acknowledged gratefully.

REFERENCES

1. Andrews, H. L. & B. L. Horecker. A simplified photo-electric colorimeter for blood analysis. Rev. Sci. Instr. In press.

2. Douglas, C. G., J. S. Haldane & J. B. S. Haldane. The laws of combination of haemoglobin with carbon monoxide and oxygen. J. Physiol., 44, 275, 1912.

3. Forbes, W. H., F. Sargent & F. J. W. Roughton. The rate of carbon monoxide uptake by normal men. Amer. J. Physiol., 143, 594, 1945.

4. Fugitt, C. H., & J. L. Lilienthal, Jr. Test of the NBS method for analysis of CO in gas mixture. Naval School of Aviation Medicine, Memorandum Report, 26 September 1944.

5. Haldane, J. B. S. The dissociation of oxyhaemoglobin in human blood during partial CO poisoning. J. Physiol., 45, 22P, 1913.

6. Helmholz, H. F., Jr., J. B. Bateman & W. M. Boothby. The effects of altitude anoxia on the respiratory processes. J. Aviat. Med. 15, 366, 1944.

7. Lilienthal, J. L., Jr., & M. B. Pine. The effect of varying partial pressure of oxygen and carbon monoxide on the uptake of carbon monoxide in man. Naval School of Aviation Medicine, Research Project X-129, #1, 15 October 1943.

8. Lilienthal, J. L., Jr., & C. H. Fugitt. The effect of low concentrations of carboxyhemoglobin upon the "altitude tolerance" of man. Naval School of Aviation Medicine, Research Project X-129, #2, 6 March 1944.

9. McFarland, R. A., F. J. W. Roughton, M. H. Halperin & J. I. Niven. The effects of carbon monoxide and altitude on visual thresholds. J. Aviat. Med., 15, 381, 1944.

10. Pace, N., W. V. Consolazio, G. C. Pitts & L. J. Pecora. The rate of blood absorption of low concentrations of carbon monoxide in ambient air at simulated altitudes up to 10,000 feet. Naval Medical Research Project X-417, #1, 25 August 1944.

11. Peters, J. P. & D. D. Van Slyke. Quantitative Clinical Chemistry, Vol. I, Interpretations, Baltimore, 1932, Williams & Wilkins Co.

12. Riley, R. L. A direct method for determination of oxygen and carbon dioxide tensions in blood. Naval School of Aviation Medicine, Research Project X-450, #2, 16 July 1945.

13. Roughton, F. J. W. & R. C. Darling. The effect of carbon monoxide on the oxyhemoglobin dissociation curve. Amer. J. Physiol., 141, 17, 1944.

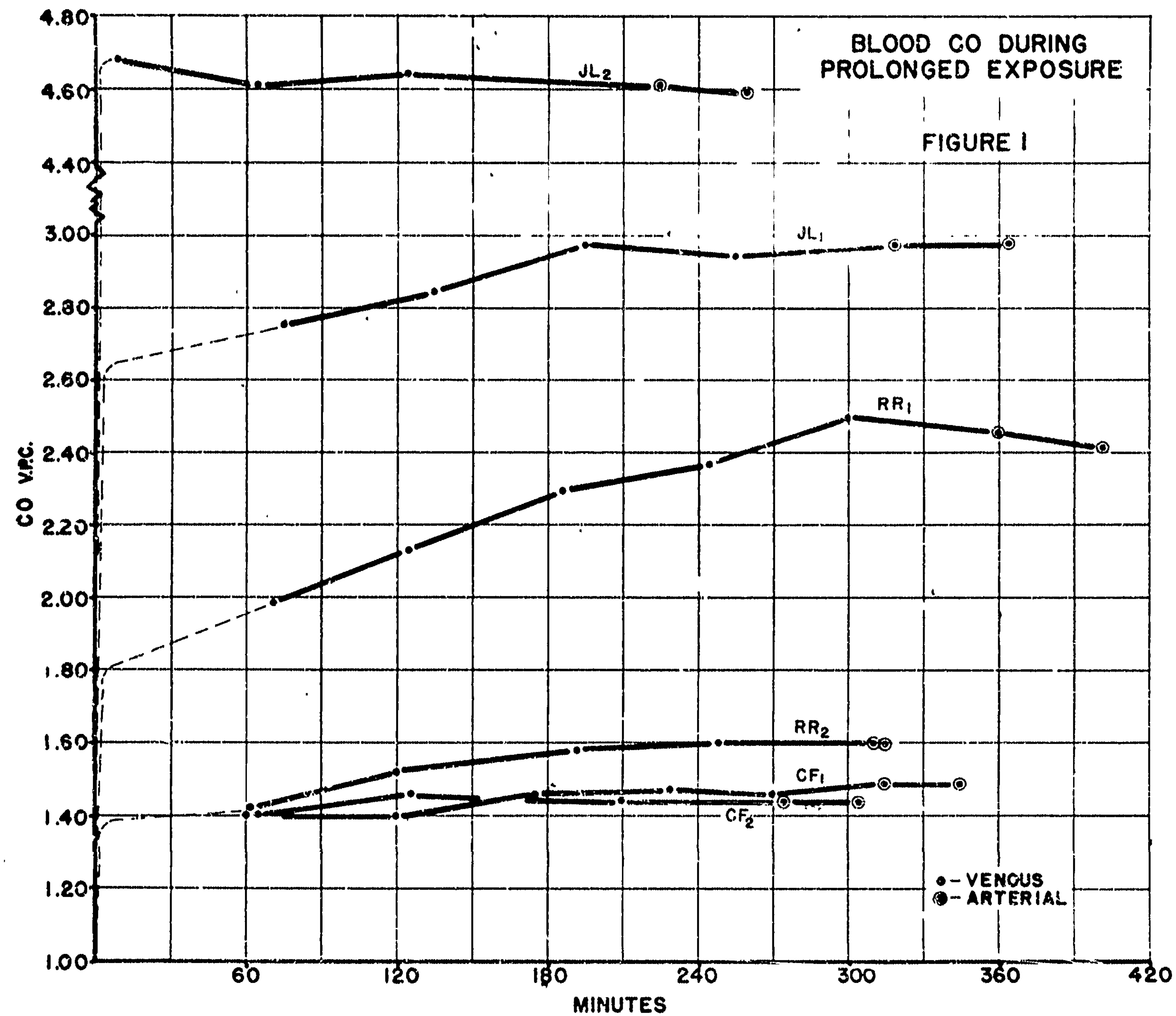
14. Sendroy, J., Jr., S. H. Liu & D. D. Van Slyke. The gasometric estimation of the relative affinity constant for carbon monoxide and oxygen in whole blood at 38°. Amer. J. Physiol., 90, 511, 1929.

15. Vollmer, E. P., B. G. King, M. B. Fisher, & J. F. Birren. The effects of carbon monoxide on three types of performance at simulated altitudes of 10,000 and 15,500 feet. Naval Medical Research Institute, Research Project X-417, #7, 27 February 1945.

TABLE I

BLOOD & GAS STUDIES DURING CO EQUILIBRIUM

SUBJECT & EXPT NO.	MINUTES OF EXPOSURE	EQUIVALENT ALTITUDE (THOUSANDS OF FEET)	INSPIRED pO_2 mm Hg	INSPIRED PER CENT CO	ARTERIAL pO_2 mm Hg	ARTERIAL pCO mm Hg	M or $\frac{pO_2 \times (CO)}{pCO \times (O_2)}$	$MpCO$ or $pO_2 \times \frac{(CO)}{(O_2)}$	$pO_2 + MpCO$	ARTERIAL O_2 CONTENT V.P.C.	ARTERIAL O_2 CAPACITY V.P.C.	ARTERIAL O_2 SATURATION V.P.C.	ARTERIAL CO CONTENT V.P.C.	ARTERIAL CO CAPACITY V.P.C.	ARTERIAL CO SATURATION V.P.C.	ARTERIAL O_2+CO CONTENT V.P.C.	ARTERIAL O_2+CO CAPACITY V.P.C.	ARTERIAL O_2+CO SATURATION V.P.C.	$\frac{100 \times (O_2Hb)}{(O_2Hb)+(COHb)+(RED.Hb)}$	ARTERIAL pCO_2 mm Hg	ARTERIAL CO_2 CONTENT V.P.C.	pH_S
CF_1	315	10.0	108	0.005	56	0.024	214	5.1	61.1	16.4	18.5	88.6	1.49	20.0	7.5	17.9	20.0	89.5	82.5	33	46.8	7.485
	345	10.0	108	0.005	59	0.024	224	5.3	64.3	[illegible]	18.5	89.2	1.49	20.0	7.5	18.0	20.0	90.0	80.6	36	46.9	7.470
CF_2	275	10.0	109	0.005	54	0.025	192	4.8	58.8	16.2	18.6	37.2	1.44	20.1	7.2	17.7	20.1	87.8	80.6	36	46.4	7.440
	305	10.0	109	0.005	58	0.025	204	5.1	63.1	16.3	18.3	89.0	1.44	19.7	7.3	17.8	19.7	90.0	82.6	33	46.6	7.450
JL_1	320	15.4	88	0.010	40	0.048	182	8.7	48.7	13.8	17.4	79.3	2.97	20.4	14.7	16.8	20.4	82.3	67.7	37	44.7	7.470
	365	15.4	88	0.010	40	0.048	184	8.7	48.7	13.7	16.7	82.0	2.97	19.7	15.2	16.7	19.7	84.7	69.5	29	44.0	7.510
JL_2	227	6.5	126	0.015	69	0.106	202	21.8	90.8	14.5	14.7	98.5	4.61	19.3	23.8	19.1	19.3	98.9	75.1	36	48.3	7.415
	260	6.5	126	0.015	66	0.106	208	21.4	87.4	14.2	14.9	95.7	4.39	19.5	23.6	18.8	19.5	96.7	78.0	38	48.1	7.405
RR_1	360	10.0	107	0.010	64	0.056	197	11.0	75.0	14.3	14.9	96.2	2.45	17.3	14.2	16.8	17.3	96.8	82.6	35	47.0	7.430
	400	10.0	107	0.010	63	0.056	191	10.6	73.6	14.3	14.9	96.2	2.41	17.3	13.9	16.7	17.3	96.5	82.6	32	47.2	7.440
RR_2	310	10.0	109	0.005	54	0.025	225	5.7	59.7	15.1	17.0	38.8	1.60	18.6	8.6	16.7	18.6	89.7	81.3	37	46.7	7.415
	313	10.0	109	0.005	54	0.025	223	5.7	59.7	15.2	17.0	89.4	1.60	18.6	8.6	16.8	18.6	90.3	81.7	40	47.1	7.415


BLOOD CO DURING
PROLONGED EXPOSURE
FIGURE I
JL2
JL1
RR1
RR2
CF1
CF2
• - VENOUS
◉ - ARTERIAL
CO V.P.C.
4.80
4.60
4.40
3.00
2.80
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
1.00
60
120
180
240
300
360
420
MINUTES

THE HALDANE EFFECT