The Hematological Effects of Chronic, Low Level Exposures to Carbon Monoxide in Rats

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In considering the toxicological potential of carbon monoxide in the atmosphere, it must be realized that only exposures to low levels, intermittently, but chronically are those realistically encountered. One speculation concerning such exposures has been the prospective elevation of red matter of the blood stimulated by the chronic hypoxemia. Such polycythemia would increase blood viscosity and impose a greater workload on the myocardium, increase resistance to blood flow, and enhance risks of thrombus formation (BURTON et al. 1969).

It is well documented that hypobaric hypoxemia in a number of mammalian species produces polycythemia characterized by reticulocytosis (BILLINGS et al., 1969; MYLEREA and ABBRECHT, 1970; SANCHEZ et al., 1970; and FINNE, 1971). The question of hematological responses to the anemic hypoxemia characterizing inhalation of carbon monoxide has not been as resolvable. RAMSEY, (1969a) has shown that brief exposures to high concentrations of carbon monoxide (1200 ppm) in rats produce significant elevations in reticulocyte percentage and hemoglobin content. RAMSEY (1969b) likewise found elevated hemoglobin content and hematocrit in human subjects exposed a few times to relatively high concentrations (650-800 ppm).

Results appear to be discrepant in respect to exposure to low levels of carbon monoxide ($<$ 500 ppm). JONES et al. (1971) obtained elevations in mean hemoglobin content and hematocrit in dogs, rats, and monkeys exposed continuously for 90 days to 100-200 ppm carbon monoxide, and WILKS et al. (1959) obtained similar results in dogs. However, MUSSELMANN et al. (1959), LINDENBERG et al. (1961), and STUPFEL and BOULEY (1970) failed to obtain significant increases in red cell mass in rabbits, rodents, and dogs exposed chronically to very low levels of carbon monoxide.

This study was an effort to determine if chronic, intermittent exposures to relatively low levels of carbon monoxide produce significant and irreversible polycythemia in rats.

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 5 © 1975 by Springer-Verlag New York Inc.

METHODS

Animal selection. One hundred and thirty post-pubertal, male, albino rats weighing approximately 300g each were used in this study. There was no significant difference in weights between the controls and exposed groups ($P \leq 0.05$).

Exposure procedure. The rats were divided randomly into 2 groups. A group of 70 (16-18 at a time) underwent exposure for 5 hrs per day for 20 days in an exposure chamber perfused with 250 ppm carbon monoxide. A second group of 60 were exposed in the very same way except that the chamber was perfused with air only. Concentrations of carbon monoxide in the chamber were monitored by the potassium-palladosulfite method.

Blood extraction. Hematological determinations were done from microquantities of blood, and blood extraction throughout the 20 days was done every other day per animal. Seven tenths ml of blood was taken from the tail vein.

Hematological evaluation. The parameters monitored included red blood cell counts with a Coulter electronic counter, hemoglobin content by the cyanmethemoglobin method, hematocrit with an International microcapillary centrifuge and reader, and reticulocyte counts from thin blood smears stained with a saturated solution of methylene blue (color index 927). In addition, values for mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentration were calculated from the measurements of red cell counts, hemoglobin content, and hematocrit.

Additional determinations. Hematological determinations were continued in both the exposed and control groups for an additional 10 postexposure days in order to evaluate reversibility of potential changes. Plasma volume determinations were performed on 10 rats. Since 3 ml of blood was needed for this, these rats were not used for other hematological determinations. The Evans dye method (T-1824) was employed. Blood was obtained by cardiac puncture upon removal from the chamber. Because of the volume of blood extracted and the dye retention, blood extraction was restricted to 10 days prior to exposure to carbon monoxide, and to the first, tenth, and twentieth days of exposure.

In order to account for the percentage saturation of the blood with carbon monoxide, 0.5 ml of blood was extracted from one of the animals every fourth day and the carbon monoxide content determined by the method of TRINDER and HARPER (1962).

RESULTS

Carboxyhemoglobin. The mean percent saturation of blood with carbon monoxide for control rats throughout 30 days was 1.02 (S.D. ± 0.32). For exposed rats the mean was 23.4% (S.D. ± 3.0).

These rats had a mean of 1.10% (S.D. ± 0.46) for the 10 days of postexposure.

Hematological response. An example of a comparison of controls and exposed rats for the 30 day time course is shown for reticulocyte percentage in Figs. 1 and 2. A comparison of controls and exposed groups for overall means in all hematological parameters during the 20 days of rat exposures is given in Table 1. All parameters with the exception of mean cell volume show a significant mean increase in exposed animals.

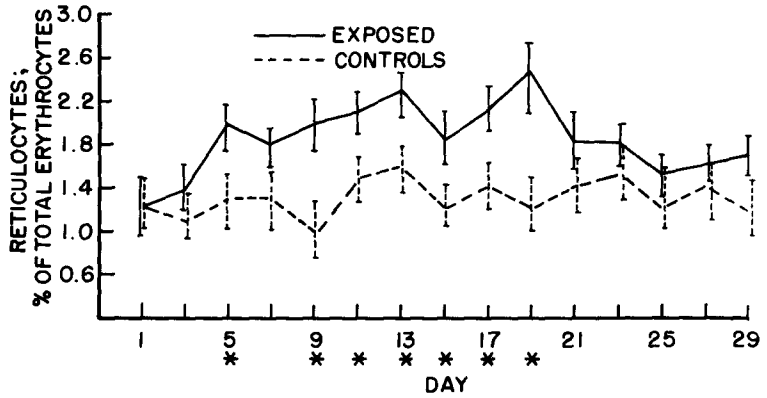

Fig. 1. Odd-day time course of means for reticulocyte percentage in rats exposed and unexposed 5 hrs/day to 250 ppm carbon monoxide for 20 days.

* Indicates exposed mean is significantly elevated above control mean.

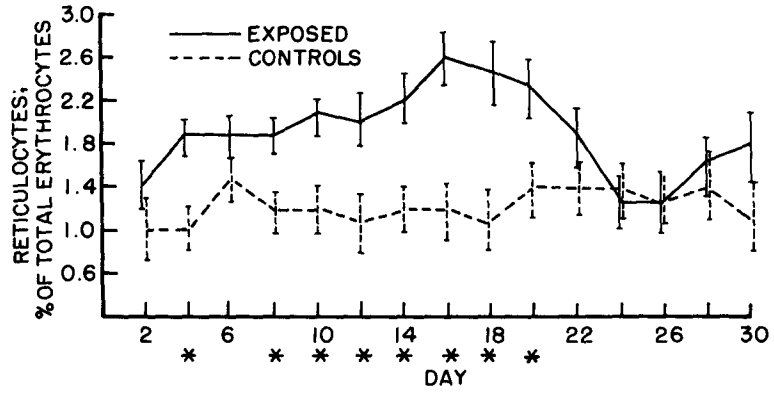

Fig. 2. Even-day time course of means for reticulocyte percentage in rats exposed and unexposed 5 hrs per day to 250 ppm carbon monoxide for 20 days.

* Indicates exposed mean is significantly elevated above control mean.

TABLE 1.

Overall means for hematological parameters in rats during 20 days of intermittent exposure to 250 ppm carbon monoxide. (The control mean and exposed mean each represent 600 measurements and 60 rats.)

	Control Mean	Exposed Mean	Significance (t-Test)
R.B.C. Count $10^6/mm^3$	8.35 ± 0.11	8.55 ± 0.27	P = <0.01
Hemoglobin Content g/100 ml	16.0 ± 0.26	16.9 ± 0.38	P = <0.001
Hematocrit %	51.1 ± 1.03	52.2 ± 0.93	P = <0.01
Reticulocytes % R.B.C.	1.23 ± 0.17	2.00 ± 0.35	P = <0.001
Mean Cell Volume μ^3	61.4 ± 1.36	60.4 ± 1.81	P = 0.02
Mean Cell Hemoglobin μμ grams	19.2 ± 0.59	19.8 ± 0.71	P = 0.05
Mean Cell Hemoglobin Concentration Vol. % of R.B.C.	31.5 ± 0.72	32.5 ± 0.40	P = <0.001

TABLE 2.

Overall means for hematological parameters in rats during 10 post exposure days following exposure to 250 ppm carbon monoxide. (The control mean and exposed mean each represent 300 measurements and 60 rats.)

	Control Mean	Exposed Mean	Significance (t-Test)
R.B.C. Count $10^6/mm^3$	8.12 ± 0.16	8.02 ± 0.164	P = 0.06
Hemoglobin Content g/100 ml	15.8 ± 0.46	16.2 ± 0.36	P = <0.01
Hematocrit %	51.4 ± 0.93	51.6 ± 0.50	P = 0.40
Reticulocytes % R.B.C.	1.30 ± 0.13	1.60 ± 0.21	P = <0.001

However, the elevations tend to become reversed during the 10 days of postexposure (Table 2). This is also well indicated for reticulocytes after day 20 in Figs. 1 and 2.

Plasma volume. The 10 rats used for plasma volume determinations showed a mean value of 6.92 ml 10 days prior to the exposure period. After the first day of carbon monoxide in the chamber the mean plasma volume was 6.49 ml; after the 10th day it was 6.54 ml, and it was 6.58 ml after the 20th day. An analysis of variance of these four means indicated no significant differences among them ($F = 0.584$).

DISCUSSION

Since the type of carbon monoxide exposures realistic for man are usually chronic, intermittent, and low level, the results of these experiments may be pertinent in establishing whether or not such exposures are capable of producing significant polycythemia. Even though there are statistically significant elevations in proportional red cell number and packed cell volume in the rat, the extremely mild degree of polycythemia should not warrant concern over potential increases in blood viscosity.

As suggested by the reticulocytosis, the increase in red cell mass is probably erythropoietic. This is further substantiated by the fact that plasma volume does not appear to change significantly. Reduction in plasma volume has been shown to be characteristic of hypobaric hypoxemia (REISSMAN, 1951; and STICKNEY and VAN LIERE, 1953).

CONCLUSIONS

Chronic, intermittent exposures to low levels of carbon monoxide (23% COHb) produce statistically significant elevations in red blood cell count, hematocrit, hemoglobin content, and reticulocyte percentage in rats. However, during ten postexposure days the elevations show significant reversibility. Because no significant change in plasma volumes appears to occur, the mild polycythemia is evidently due to increased erythropoiesis.

The degree of polycythemis obtained is probably not critical in respect to increases in blood viscosity.

ACKNOWLEDGEMENTS

This study was supported by The National Science Foundation Institutional Grant Program of the University of Dayton (Ohio) Research Council. Michael Stablein, William Turnbull, and Thomas Fitzsimmons provided technical assistance.

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