Nitrosylhemoglobin and Carboxyhemoglobin in the Blood of Mice Simultaneously Exposed to Nitric Oxide and Carbon Monoxide

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We have reported previously (ODA et al. 1975a) that nitrosylhemoglobin (NOHb) was formed in the blood of animals exposed to nitric oxide (NO) and that this action of NO to hemoglobin is similar to the well-known action of carbon monoxide (CO). It would therefore be advantageous to make a comparative study of NO to CO in the toxicological study on NO. Furthermore, as these gases are contained in the automobile exhaust gas, a study on their combined effects would contribute much toward the elucidation of the hazardous effects of automobile exhaust gas on the human body.

With regard to the effect of simultaneous exposure to CO and other gases, MURPHY (1964) has shown that the blood concentration of carboxyhemoglobin (COHb) was higher after simultaneous exposure to CO at 300 ppm with ozone at 0.75 ppm than after exposure to CO at 300 ppm alone. LUTMER et al. (1967) have reported that there was no difference in blood concentration of COHb between exposure to CO at 120 ppm alone and simultaneous exposures to CO at 120 ppm with NO at 3 ppm, with NO_2 at 3 ppm, or with ozone at 0.75 ppm. Furthermore NAKAJIMA and KUSUMOTO (1970) could not show any difference in COHb between exposure to CO at 50 ppm with NO_2 at 0.2-0.8 ppm and exposure to CO at 50 ppm alone.

The reports cited above were all concerned with the level of COHb only, but in the present study the concentration of NOHb in the blood as well as COHb was determined in order to elucidate the interaction of NO and CO in hemoglobin binding. In addition, some mention will be made of recent advances in the method for NOHb determination by electron spin resonance (ESR) spectrometer-computer on-line system.

MATERIALS AND METHODS

The animals used in this study were 8 weeks old JCL-ICR strain female mice and blood specimens were drawn from the orbital vein immediately after exposure.

Bulletin of Environmental Contamination & Toxicology, Vol. 16, No. 5 © November 1976 by Springer-Verlag New York Inc.

Five to 10 mice were used in each group of the experiment.

Animal exposure system to NO using pure NO gas in a cylinder and determination of the concentrations of NO and NO_2 were made as previously reported (ODA et al. 1975a). The animals were exposed to NO at the concentrations of 9.5 ppm (11.7 mg/m^3) for 180 minutes and 66 ppm (81 mg/m^3) for 90 minutes, while contamined NO_2 concentrations were 1.8 ppm (3.4 mg/m^3) and 36 ppm (68 mg/m^3), respectively.

For CO exposure, pure CO gas from a cylinder was diluted with air to the specified concentrations and led into the exposure chamber. CO concentration was determined by means of a non-dispersion type infrared spectrometer (Hitachi-Horiba CO analyzer, APMA-10). The animals were exposed to CO at the concentrations of 100 ppm (115 mg/m^3) for 180 minutes and 460 ppm (528 mg/m^3) for 90 minutes.

In the exposure to the mixtures of NO and CO, the animals were exposed to the mixtures of 9.5 ppm NO and 100 ppm CO for 180 minutes (low level), and 66 ppm NO and 460 ppm CO for 90 minutes (high level). The variance of gas concentration during exposure was maintained within 10 percent. Control animals were fed in a filtered clean room air.

NOHb in the blood of the animals was detected by X-band ESR spectrometer (JES-PE-3X, JEOL LTD.) at -140°C as previously reported (ODA et al. 1975a). In the present study NOHb contents were calculated by an on-line data processing system, as shown in Fig. 1.

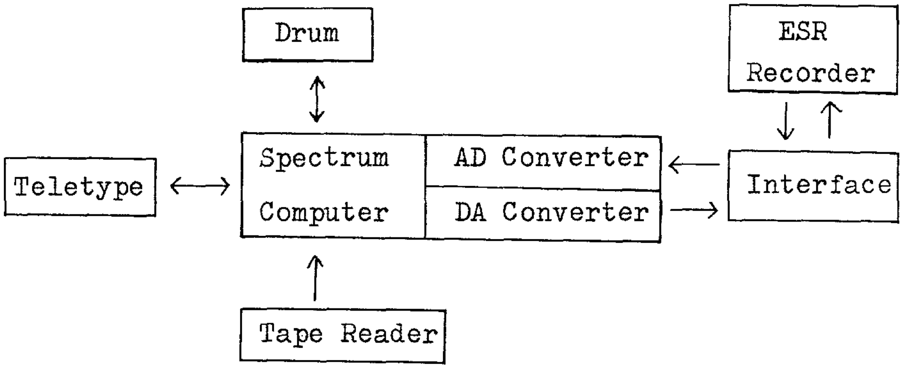

Fig. 1 Block diagram of the computer system for quantitative determination of NOHb.

Analog data obtained by the spectrometer were converted to digital data by analog-digital converter built in the minicomputer (JEC-6, JEOL LTD.). The area of the spectrum was calculated by two times integrations and then was converted to NOHb contents. The program also included accumulative operation to improve the signal-noise ratio, operation to correct the incline of base line and tabulation of the data. There was a high correlation (r=0.98) between the spectral area calculated by this method and the spectral amplitude read conventionally on the chart.

COHb was measured mainly by Kampen's method (VAN KAMPEN and ZIJLSTRA, 1965), but because of the possibility that this spectrophotometric procedure might be influenced by the presence of NOHb, modified Lawther's method (LAWTHER and APTHORP, 1955) was also employed for cross-checking of the determination of COHb after exposure of the animals to CO at 460 ppm and a mixture of 66 ppm NO and 460 ppm CO. In the latter method, CO, liberated from about 0.5 ml of blood in deoxygenated 0.1 % ammonia solution by adding potassium ferricyanide, saponin and lactate, was trapped in a one liter vacuum bottle and then introduced to the CO analyzer.

Total hemoglobin was determined by the cyanmethemoglobin method after hemolysis and dilution of a part of the blood sample.

RESULTS

Though NOHb was not detected in the control animals kept in clean air, the blood of mice exposed to NO showed an ESR spectrum of NOHb. The average blood concentration of NOHb was 0.15 % in the 9.5 ppm NO group and 1.58 % in the 66 ppm NO group as shown in Table 1. As previously reported the blood concentration of NOHb was dependent on the concentration of NO to which the animals were exposed.

COHb contents shown in Table 1 were determined by Kampen's method. In the determination of COHb in exposure to CO at 460 ppm alone and exposure to CO at 460 ppm with NO at 66 ppm both Kampen's and Lawther's methods were employed, but as shown in Table 2 there was no significant difference between the results obtained by both methods.

The NOHb and COHb contents of mice exposed simultaneously to NO and CO did not show any significant difference from separate exposures in both low level and high level of gases as can be noted in Table 1.

TABLE 1

Contents of NOHb and COHb determined immediately after exposure to NO, CO and their mixture (m ± SD)

		NOHb %	COHb %
Groups exposed to clean air		ND	ND
Groups exposed	NO 9.5 ppm	0.15 ± 0.04	ND
to low level	CO 100 ppm	——	12.1 ± 2.3
of gases	NO 9.5 ppm + CO 100 ppm	0.13 ± 0.04	11.8 ± 3.5
Groups exposed	NO 66 ppm	1.55 ± 0.36	ND
to high level	CO 460 ppm	——	30.8 ± 3.4
of gases	NO 66 ppm + CO 460 ppm	1.68 ± 0.45	27.7 ± 2.5

ND: NOHb < 0.01 % , COHb < 0.1 %   —— : not measured

TABLE 2

Comparison of COHb contents determined by Kampen's and Lawther's method (m ± SD , %)

	CO 460 ppm	NO 66 ppm + CO 460 ppm
Kampen's method	30.8 ± 3.4	27.7 ± 2.5
Lawther's method	30.8 ± 2.0	31.2 ± 2.9

DISCUSSION

In the previous study the quantitative determination of NOHb by ESR was made by reading the amplitude of spectrum. In the present study the ESR spectrometer was combined with a computer, making it possible to obtain a secondary integral of the ESR spectrum, and the blood concentrations of NOHb could be determined with high accuracy. A high correlation between the spectrum area obtained by this method and the spectrum amplitude read on the chart by means of the conventional method suggests that the conventional method can reasonably be used as an expedient.

ANTONINI and BRUNORI (1971) have reported that the affinity of NO to hemoglobin was 1400 times higher than the affinity of CO to hemoglobin *in vitro*. On the basis of their data we speculated that only 0.4 ppm NO may be able to saturate half of the hemoglobins in the blood (ODA et al. 1975b). As shown in the present study, however, the blood concentration of NOHb was 1.55 % in the animals exposed to NO at 66 ppm, whereas that of COHb was 12.1 % in the animals exposed to CO at 100 ppm. This shows that the formation of NOHb in the body is much lower than calculated from the ambient gas concentrations on the basis of *in vitro* data and also lower than COHb contents formed by exposure of CO at the same level. If the *in vitro* data, though obtained in the absence of oxygen, can be applied to living animals, the results obtained in this study suggest that almost none of the NO gas inhaled reaches hemoglobin, or it may change to other substances such as NO_2 and nitrite. Therefore, the fate of NO in the body should be studied in detail and the isotope method using ^{15}NO (FREEMAN and ANBAR 1973) would be useful for such a study.

It is not reasonable to compare the toxicity of NO and CO on living body only on the basis of their blood concentrations of NOHb and COHb. It is important to collect data to permit comparison of other effects of NO and CO on living body.

The present study showed no difference in the contents of NOHb and COHb between simultaneous exposure and separate exposure of NO and CO. These results suggest that NO and CO exhibit little or no interaction on hemoglobin binding at the exposure concentrations used in this experiment. From another point of view, these experimental results also suggest that the effects of NO and CO on the contents of NOHb and COHb can be determined independently of one gas to another in the situation where CO and NO coexist in pollution by automobile exhaust gases. However, as NO and CO may have an additive or synergic effects on other organs, the combined effects should be studied further in detail.

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