Biochemical Pharmacology, Vol. 26, pp. 557–558. Pergamon Press, 1977. Printed in Great Britain.

Effect of dichloromethane on hemoglobin function

(*Received* 24 *May* 1976; *accepted* 23 *July* 1976)

An increase in blood carboxyhemoglobin saturation (COHb) was observed when humans [1, 2], rats [3] and rabbits [4] were exposed to dichloromethane vapor. Biological conversion of isotopically labeled CH_2Cl_2 to carbon monoxide (CO) has been demonstrated by Kubic *et al.* [5], by Zorn [6] and by DiVincenzo and Hamilton [7]. Recently Nunes and Schoenborn [8] found that CH_2Cl_2 binds to myglobin. Binding of CH_2Cl_2 to hemoglobin has been reported by Settle [9]. These authors suggest that the increase in COHb observed when animals are exposed to CH_2Cl_2 is due to a change in the affinity of the hemoglobin for CO instead of an increase in the total amount of CO present. This study was done to determine the effect of CH_2Cl_2 on the relative affinity of hemoglobin for oxygen and CO.

Fresh samples of EDTA-treated human and rat blood were used. The blood was equilibrated with air containing added CO alone. Another sample of the same blood was equilibrated with air containing 1% (v/v) CH_2Cl_2 vapor to which a similar amount of CO was added. A double tonometer [10] was used in which the whole blood sample (4 ml) was spread as a thin film on the walls of the 350-ml gas tonometer by continuous rotation. Equilibration was for 2 hr in a 37° water bath. Light was excluded by wrapping the tonometer system in aluminum foil. When equilibrium was reached, the blood was allowed to flow into the small tonometer and the blood and gas phases were separated, without exposure to air, at the water bath temperature. The equilibrated blood was analyzed for total hemoglobin [11], CO content [12, 13] and methemoglobin (MetHb) [14]. The equilibrium gas phase was analyzed for oxygen by O_2 electrode and for CO by gas chromatography [15]. All analyses were done in duplicate.

Oxygen in the gas phase (always greater than 20% by volume) was sufficient to oxygenate essentially all of the hemoglobin except that present as COHb or MetHb. Hence, the Haldane relative affinity constant, K, was calculated from the relation:

$$K = \frac{(COHb)}{(O_2Hb)} \times \frac{(O_2)}{(CO)}$$

where $(COHb)$ is the fraction of the total Hb combined with CO, and (O_2Hb) is the fraction of the total Hb combined with oxygen, that is $(O_2Hb) = 1(MetHb)-(COHb)$. No sample of blood used exceeded 0.4% of the total hemoglobin as MetHb. The symbols (O_2) and (CO) represent per cent by volume of the dry equilibrium gas phase present as oxygen and as carbon monoxide. For both species, the range of equilibrium (CO) concentration was from 0.01 to 0.1%, i.e. 100 to 1000 ppm.

The results obtained are summarized in Table 1. The five samples of human blood gave a mean value of $K_{37°} = 227 \pm 2.9$ (S. E. M.) in the absence of CH_2Cl_2.

Table 1. Effect of dichloromethane on the Haldane relative affinity constant of hemoglobin for CO and O_2

Species and sample	Equilibrium gas composition* CH_2Cl_2 (%)	O_2 (%)	CO (%)	Equilibrium blood ratio (COHb)/(O_2Hb)	Haldane constant ($K_{37°}$)
Human					
1	0	20.9	0.0264	0.274	217
1	1	20.8	0.0313	0.329	219
2	0	20.8	0.0304	0.332	227
2	1	20.9	0.0715	0.780	228
3	0	20.9	0.0995	1.089	229
3	1	20.9	0.0766	0.852	232
4	0	20.9	0.0083	0.091	229
4	1	20.9	0.0082	0.090	229
5	0	20.9	0.0087	0.098	235
5	1	20.9	0.0106	0.116	229†
Rat					
1	0	20.9	0.0262	0.224	179
1	1	20.8	0.0202	0.171	176
2	0	20.9	0.0428	0.364	178
2	1	20.8	0.0225	0.189	175
3	0	20.9	0.0240	0.203	177
3	1	20.8	0.0415	0.350	175
4	0	20.9	0.0217	0.185	178
4	1	20.8	0.1165	0.997	178‡

* Gas concentrations are expressed as % by volume of dry gas.
† Human blood: $N = 10$, mean ± S. E. M., $K_{37°} = 227 \pm 1.7$.
‡ Rat blood: $N = 8$, mean ± S. E. M., $K_{37°} = 177 \pm 0.5$.

Table 2. Effect of dichloromethane on binding of CO by rat blood in nitrogen

Sample	Blood COHb (% sat.)	Gas phase O_2* (%)	Gas phase CO* (%)	Gas phase CH_2Cl_2† (%)
A	10.5			0
A	10.5	0.28	0.0071	1.0
B	33.4			0
B	33.4	0.15	0.0112	1.0

* Measured concentrations as % by volume of dry gas at equilibrium.

† Initial value after CH_2Cl_2 addition.

Addition of 1% by volume of CH_2Cl_2 vapor to the gas phase gave a value of $K_{37} = 227 \pm 2.2$. There is no difference between these values when tested by the Student t-test. $P > 0.9$. Analysis of four samples of pooled rat blood gave a control value of $K_{37} = 178 \pm 0.4$ which was not significantly different, $P > 0.1$, from the value of $K_{37} = 176 \pm 0.7$ observed in the presence of CH_2Cl_2. The data clearly show that dichloromethane in amounts up to 1% in the gas phase does not change the relative binding of oxygen and CO by hemoglobin in either species.

Two samples of rat blood were used to test the effect of CH_2Cl_2 on CO binding in the absence of oxygen. Samples of blood were first equilibrated as above for 2 hr with CO-nitrogen mixtures. At this time, a portion of the blood was removed for analysis without opening the system to air. Liquid CH_2Cl_2 (8 μl) was then injected into the tonometer and allowed to vaporize. This amount of methylene chloride vapor (0.13 m-mole) is sufficient to provide 1% by volume of CH_2Cl_2 vapor in the 350-ml gas tonometer. The closed system was then re-equilibrated for an additional hr at 37° to note any change in COHb saturation. The results given in Table 2 show no change in COHb saturation at either level of CO when the CH_2Cl_2 was added.

The present data show that neither the relative affinity of hemoglobin for carbon monoxide and oxygen nor the absolute affinity for CO, measured in the absence of oxygen, is altered by the presence of CH_2Cl_2. The suggestion of Settle [9] that the increase in COHb caused by dichloromethane could be due to a change in CO binding affinity is not supported by these data. Such increases in COHb are more likely due to increased biological formation of CO in the presence of CH_2Cl_2. An increase in CO production by rats to greater than 30 times the endogenous level has been observed* in the presence of CH_2Cl_2 at a dose of 0.1 to 1.0 m-mole/kg.

In summary, the relative affinity (Haldane constant) for carbon monoxide and oxygen of human and rat hemoglobin was measured for whole blood *in vitro*. Equilibrations at 37° were performed with and without dichloromethane in the gas phase. Control results for human blood, Haldane constant $K_{37} = 227 \pm 2.9$ (S. E. M.), were unchanged in the presence of 1% CH_2Cl_2 vapor in the gas phase. Similar amounts of dichloromethane in the presence of rat blood gave results the same as the control value for this species, $K_{37} = 178$. Dichloromethane did not alter the binding of CO by rat hemoglobin in the absence of oxygen. These results indicate that CH_2Cl_2 does not change the absolute affinity of hemoglobin for CO nor its relative affinity for CO and oxygen. Elevation of COHb observed when man or animals are exposed to CH_2Cl_2 is due to an increase in the rate of CO production rather than a change in the CO-hemoglobin affinity.

* Unpublished observations from this laboratory.

Acknowledgements—This work was supported by the Naval Medical Research and Developmental Command, National Naval Medical Center, Department of the Navy, Research Task MR041.01.01.0103. The opinions or assertions contained herein are those of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

Laboratory of Analytical Biochemistry, Environmental Biosciences Department, Naval Medical Research Institute, Bethesda, Md. 20014, U.S.A.

HAROLD A. COLLISON
F. LEE RODKEY
JOHN D. O'NEAL

REFERENCES

1. R. D. Stewart, T. N. Fisher, M. J. Hosko, J. E. Peterson, E. D. Baretta and H. C. Dodd, *Science, N.Y.* **176,** 295 (1972).
2. R. D. Stewart, T. N. Fisher, M. J. Hosko, J. E. Peterson, E. D. Baratta and H. C. Dodd, *Archs envir. Hlth.* **25,** 342 (1972).
3. G. G. Fodor, D. Prajsnar and H.-W. Schlipköter, *Straub: Reinhaltung der Luft* **33,** 258 (1973).
4. R. P. Roth, R. T. Drew, R. J. Lo and J. R. Fouts, *Toxic. appl. Pharmac.* **33,** 427 (1975).
5. V. L. Kubic, M. W. Anders, R. R. Engel, C. H. Barlow and W. S. Caughey, *Drug Metab. Dispos.* **2,** 53 (1972).
6. H. Zorn, in *Bericht über die 14 Jahrestagung der Deutschen Gesellschaft für Arbeitsmedizin e. V.* (Eds. G. Lehnert, D. Szadkowski and H. J. Weber), p. 343. Gentner Verlag, Stuttgart (1975).
7. G. D. DiVincenzo and M. L. Hamilton, *Toxic. appl. Pharmac.* **32,** 385 (1975).
8. A. C. Nunes and B. P. Schoenborn, *Molec. Pharmac.* **9,** 835 (1973).
9. W. Settle, *Fedn Proc.* **34,** 229 (1975).
10. L. S. Fridericia, *J. biol. Chem.* **42,** 245 (1920).
11. E. J. Van Kampen and W. G. Zijlstra, *Clinica chim. Acta* **6,** 538 (1961).
12. H. A. Collison, F. L. Rodkey and J. D. O'Neal, *Clin. Chem.* **14,** 162 (1968).
13. F. L. Rodkey and H. A. Collison, *Clin. Chem.* **16,** 896 (1970).
14. F. L. Rodkey and J. D. O'Neal, *Biochem. Med.* **9,** 261 (1974).
15. F. L. Rodkey, *Ann. N.Y. Acad. Sci.* **174,** (Article 1), 261 (1970).