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ENDOGENOUS CARBON MONOXIDE PRODUCTION IN ALLOXANIZED RATS

BY

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ABSTRACT

The endogenous production of carbon monoxide (Vco) in thirty-three diabetic rats induced by alloxan and in fifteen normal rats was measured by serial determinations of the expired carbon monoxide and of the increase of carbon monoxide in the blood during rebreathing in a closed system.

Vco (mean ± S.E., μl/250g/hr) in the group of alloxanized rats 24, 48, 72, 96, 120 and over 144 hours after the alloxan injection was 6.2 ± 2.9, 8.4 ± 2.1, 9.2 ± 1.8, 10.7 ± 3.5, 16.4 ± 2.0 and 16.5 ±2.2, respectively, whereas it was 5.9 ± 0.4 for the control.

This indicates that the alloxanized group maintaining a high blood sugar level for over 120 hours has a three-fold higher Vco than the control.

INTRODUCTION

In the late 1940's, it was shown by Sjöstrand[1] that carbon monoxide (CO) is produced endogenously in the normal adult during heme catabolism.

Concerning the various conditions of heme degradation (Lynch *et al.*[2], Mercke *et al.*[3]), effects of several substances on heme dynamics (Lundh *et al.*[4], Landaw *et al.*[5]) and the disorders of hematopoietic system (Coburn *et al.*[6], Maisels *et al.*[7]), numerous studies have been done to date, and the methods of accurately measuring the CO production in vivo have been developed (Coburn *et al.*[8,9], Ostrander *et al.*[10]). But, there are no studies about Vco in metabolic disorders.

On the other hand, it has been reported that the blood carboxyhemoglobin (COHb) percent concentration ([COHb]%) was increased in the patients with diabetes mellitus (Belli *et al.*[11], Crosetti *et al.*[12], Giubileo *et al.*[13] and a similar result was confirmed by our study (Takano *et al.*[14]). However, it is entirely unknown whether the origin of the increased CO in the blood is due to the endogenous production or exogenous absorption.

The aim of this preliminary report is to estimate the Vco in the alloxanized rats and compare it with that in the normal rats.

METHODS

Thirty-three male Wistar rats weighing about 250 g treated with alloxan (300 mg/kg, i.p.) and fifteen male animals also treated with 0.9% NaCl as the control were subjected to the experiment.

Fig. 1 is a schematic illustration of the closed rebreathing apparatus, in which the rat is isolated from the environmental air and made to breathe for several hours. The capacity of the circuit was 1.4 l and it was

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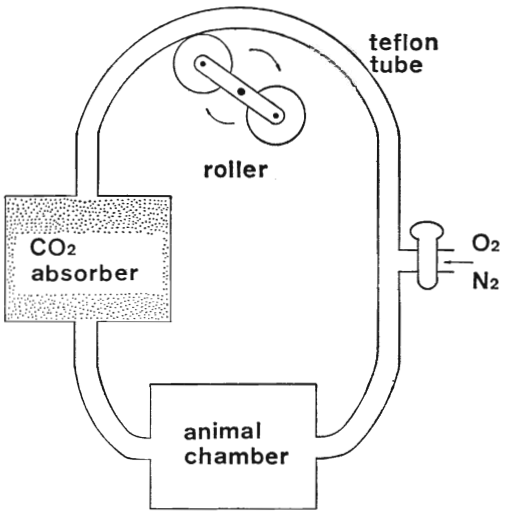

Fig. 1. Schematic Illustration of a Closed Rebreating Apparatus

filled with oxygen and nitrogen by an air bag to keep the gas volume in the circuit constant.

The experimental procedure was as follows: The rat was put in the apparatus, and after an equilibration period of 20 minutes in the rebreathing system gas samples (1 l) were drawn every 2 hours. The duration of rebreathing time varied from 2 to 8 hours. Blood CO levels before the rebreathing experiments were estimated from the two additional groups of rats under corresponding conditions.

Vco was approximated by the sum of the excreted pulmonary CO and the increased CO in the blood. The expired CO was collected in the air bag and measured by a nondispersive infrared gas analyzer. The evaluation of the CO volume percent in the blood was performed according to the method of Lawther and Aptorp[15].

Blood sugar was measured according to the method of Hyvärinen and Nikkilä[16].

Results

Fig, 2 shows the relation of Vco and the time course after the alloxan injection. Vco (mean ± S.E., μl/250/hr, at 0°C, and 0% R.H. of the atomosphere) in the group of alloxanized rats 24, 48, 72, 96, 120 and over 144 hours after the injection was 6.2 ± 2.9, 8.4 ± 2.1, 9.2 ± 1.8, 10.7 ± 3.5, 16.4 ± 2.0 and 16.5 ± 2.2, respectively, whereas it was 5.9 ± 0.4 for the control.

As can be seen in Fig. 3, the alloxanized group maintaining a high blood sugar level

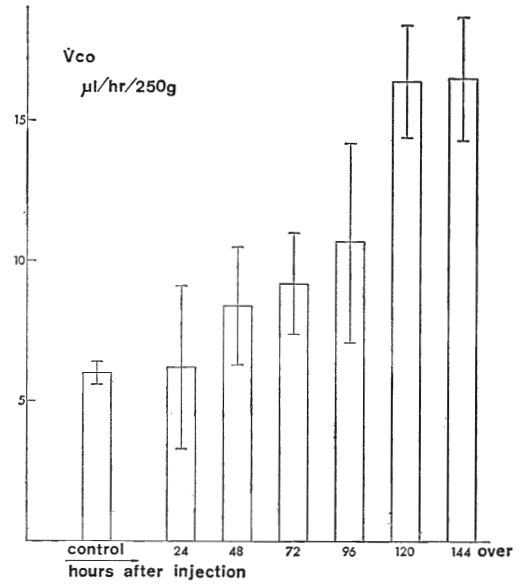

Fig. 2. Relation of Endgenous Carbon Monoxide Production (Vco) and Time Course After Alloxan Injection

Table 1. Endogenous Carbon Monoxide Production (Vco μl/250 g Body Weight): Control Rats and Alloxanized Rats Over 120 Hours

Rebreathing hours	Vco $\mu\ell$ 250 g body weight		
	Control	Alloxanized	p
2	11.9±0.7	33.2±4.2	<0.01
4	20.3±2.1	61.4± 9.9	<0.01
6	36.0±4.1	89.9±13.7	<0.01

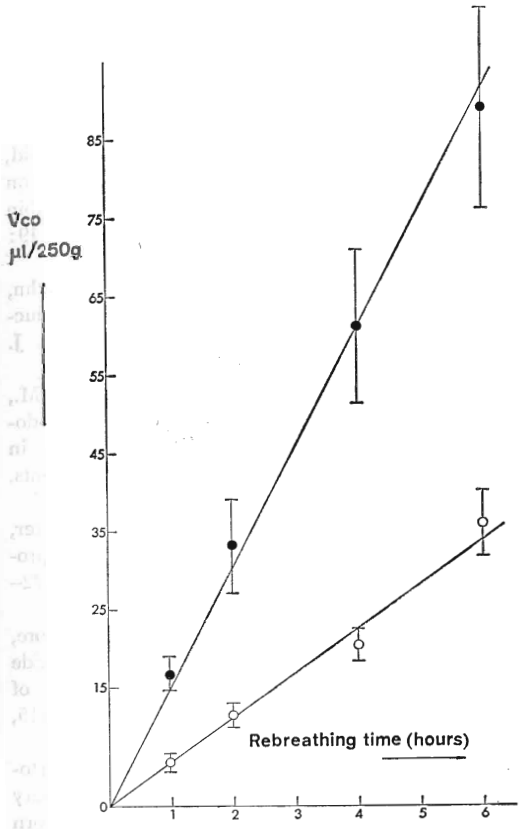

Fig. 3. Endogenous Carbon Monoxide Production (Vco) During Rebreathing: Alloxanized Rats Over 120 Hours After Injection (Filled Circles) and Control Rats (Open Circles)

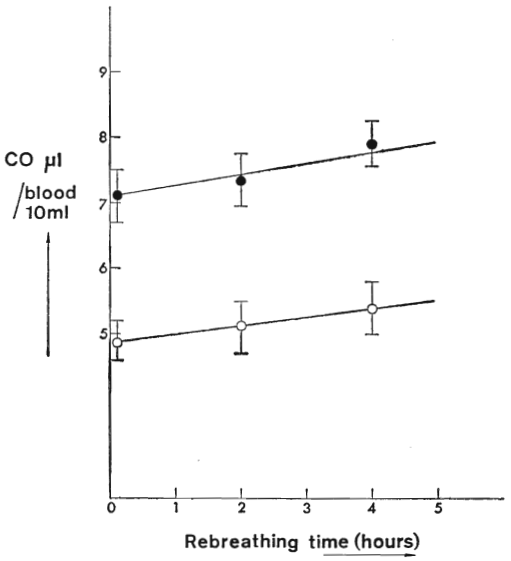

Fig. 4. Blood CO Levels Before and During Rebreathing: Alloxanized Rats Over 120 Hours After Injection (Filled Circles) and Control Rats (Open Circles)

for over 120 hours showed a significantly higher Vco than the control ($p<0.01$). Detailed figures are shown in Table 1.

As shown in Fig. 4, in both the normal and alloxanized groups, the blood CO level (mean $\pm$ S.E., μl/10ml) increased significantly by the maintainance of rebreathing, that is 4.9 ± 0.3 at 0 hour and 5.4 ± 0.4 at 4 hours after rebreathing for the control and 7.1 ± 0.4 at 0 hour and 7.9 ± 0.3 at 4 hours after rebreathing for the alloxanized group.

The blood sugar level (mean $\pm$ S.E., mg/dl) at the end of rebreathing was 455.5 ± 34.8 (ranging from 298.3 to 665.0) in the alloxanized group and 111.1 ± 2.8 in the control group. However, there was no significant correlation between the blood sugar and Vco levels.

Discussion

It was indicated in the present study that Vco is increased in the alloxanized rats compared with the normal rats.

According to the established theory that endogenous CO originates not from the other substances but from the alpha bridge carbon atom of heme (Coburn *et al.*[17]), it could be said that a certain acceleration of heme catabolism seems to occur in the diabetic rats induced by alloxan.

However, considering the fact that the alpha bridge carbon atom forms formyl once during heme degradation (Nakajima *et al.*[18]), and the publication reporting that L-dopa produces CO in the biological enzymatic reaction to melanin in vitro (Miyahara *et al.*[19]), it could be implied that there are some possibilities that there are other

metabolic pathways which produce CO in a diabetic body.

The question as to whether [COHb]% in alloxanized rats with a high Vco could reach levels associated with CO toxicity has not been completely answered. Nevertheless, there are too many places with a considerably high CO concentration because of environmental pollution, and, in addition, aortic intimal injury and/or ultrastructual myocardial damage have been found under hypoxia due to the expossure to a rather low level of CO in rabbits (Thomsen *et al.*[20], Kjeldsen *et al.*[21]). Accordingly, patients who produce endogenous CO excessively and cannot excrete this harmful waste substance promptly seem to suffer from a high risk of cardiovascular diseases due to both external and internal environmental conditions.

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References

1) Sjöstrand, T.: Endogenous formation of carbon monoxide in man under normal and pathological conditions. Scand. J. Clin. Lab. Invest., 1: 201–214, 1949.
2) Lynch, S. R., and Moede, A. L.: Variation in the rate of endogenous carbon monoxide production in normal human beings. J. Lab. Clin. Med., 79: 85–95, 1972.
3) Mercke, C., Cavallin-Stahl, E., and Lundh, B.: Carbon monoxide production and reticulocyte count in normal women. Acta med. Scand., 198: 155–160, 1975.
4) Lundh, B., Cavallin-Stahl, E., and Mercke, C.: Nicotinic acid and the the endogenous production of carbon monoxide. Acta med. Scand., 197: 173–176, 1975.
5) Landaw, S. A., Callahan, E. W., and Schmid, R.: Catabolism of heme in vivo: Comparison of the simultaneous production of bilirubin and carbon monoxide. J. Clin. Invest., 49: 914–925, 1970.
6) Coburn, R. F., Williams, W. J., and Kahn, S. B.: Endogenous carbon monoxide production in patients with hemolytic anemia. J. Clin. Invest., 45: 460–468, 1966.
7) Maisels, M. J., Pathak, A., Nelson, N. M., Nathan, D. G., and Smith, C. A.: Endogenous production of carbon monoxide in normal and erythroblastotic newborn infants. J. Clin. Invest., 50: 1–8, 1971.
8) Coburn, R. F., Blakemore, W. S., and Forster, R. E.: Endogenous carbon monoxide production in man. J. Clin. Invest., 42: 1172–1178, 1963.
9) Coburn, R. F., Danielson, G. K., Blakemore, W. S., and Forster, R. E.: Carbon monoxide in blood; Analytical method and sources of error. J. Appl. Physiol., 19(3): 510–515, 1964.
10) Ostrander, C. R., Johnson, J. D., and Bartoletti, A. L.: Determining the pulmonary excretion rate of carbon monoxide in newborn infants. J. Appl. Physiol., 40(5): 844–848, 1976.
11) Belli, R., and Givliani, V.: Ricerche sull'ossicarbonismo ossicarbone mia endogena e diagnosi di ossicarbonismo cronico. Folia Med., 38(2): 127–132, 1955.
12) Crosetti, L., Pettinati, L., and Rubino, G. F.: Alcune considerazional in tema di preuenzione tecnica e biologica dell'intossicazione da ossido di carbonio. Med. Lavoro., 56: 604–612, 1965.
13) Giubileo, M., and Parmeggiani, L.: Studio comparation sulla determinazione della HbCO e del CO estraibile dal sangue. Med. Lavoro., 50: 91–107, 1959.
14) Takano, T., Honma, K., and Maeda, H.: (in Japanese) Alloxan rat oyobi seijyo rat ni okeru naiinsei CO sanseiryo no hikaku (Comparitive study on endogenous carbon monoxide production in alloxanized rats and normal rats). Jap. J. Hyg., 31(1): 237, 1976.
15) Lawther, P. J., and Apthorp, G. H.: A method for the determination of carbon monoxide in blood. Brit. J. Industr. Med., 12: 326–329, 1955.
16) Hyvärinen, A., and Nikkilä, E. A.: Specific

determination of blood glucose with o-toluidine. Clin. Chem. Acta., 7: 143–144, 1972.
17) Coburn, R. F.: Endogenous carbon monoxide production. N. Engl. J. Med., 282(4): 207–209, 1970.
18) Nakajima, H.: Studies on heme α-methenyl to fissures as susceptible areas J. Dent. Res., 42: 461–476, 1963.
19) Miyahara, S.: Biological CO evolution. J. Biochem., 69: 231–233, 1971.
20) Thomsen, H. K., and Kjeldsen, K.: Aortic intimal injury in rabbits. Arch. Environ. Health, 30: 604–607, 1975.
21) Kjeldsen, K., Thomsen, H. K., and Astrup, P.: Effects of carbon monoxide on myocardium. Circulation Research, XXXIV: 339–348, 1974.