Influence of Carbon Monoxide Upon Some Respiratory Enzymes of the Chick Embryo

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Numerous studies have established the oxygen-carbon dioxide requirements for normal avian incubation and have shown that morphologic and enzymatic alterations occur in embryos beyond these limits (1-6). However, relatively few studies have been made on the effects of specific atmospheric gaseous pollutants upon the development and metabolic and biochemical activity of the chick embryo. The present report provides initial data on the effect(s) of carbon monoxide (CO) upon the levels of two respiratory enzymes, lactic dehydrogenase (LDH) and cytochrome oxidase (COx), of the chick embryo heart following exposure to 425 ppm CO in air.

Materials and Methods

1. Exposure chambers. Experimental chambers, flow rates, and monitoring of CO have been previously described (7).

2. Incubation of eggs and handling of embryos. Fertile eggs weighing 56.0 ± 2.4 g from a White Leghorn flock maintained at the Griffin Laboratory of the New York State Department of Health were used in these studies. Prior to exposure, all eggs began their incubation in a commercial incubator-hatcher (Jamesway, Butler Corp., Fort Atkinson, Wisconsin). Since the analyses were to be performed on 17-day-old chick embryo hearts following CO-exposure periods varying from 24 to 168 hours, embryonated eggs of known incubation age were removed from the incubator, candled, and inserted into the exposure chamber. Exposure to CO was uninterrupted for each experimental period. An equivalent number of embryonated eggs was inserted into the control chamber.

On removal from the test chambers, eggs were gently opened and individual blood samples for carboxyhemoglobin determinations (COHb) were obtained from the allantoic artery of several embryos from each group. Hearts were then removed, cleared of adhering blood and tissue material, weighed in screw capped vials and frozen at -20° C.

3. Carboxyhemoglobin determinations. Blood of both control and exposed chick embryos were analyzed with the Gilford 2400 spectrophotometer according to the method of Buchwald (8).

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4. Enzymatic determinations. A 1% tissue homogenate of the heart was prepared with cold 0.25 M sucrose. After centrifugation at 1500 rpm for 20 minutes at 4° C, the supernatant was collected. A 2-ml aliquot of the supernatant was removed for COx assays, and dilutions of 1:5 and 1:10 with 0.25 M sucrose were prepared from the supernatant for LDH determinations.

Cytochrome oxidase activity was determined according to the method of Gibbs (9) and expressed as the extinction at 540 mμ (E_{540}) per milligram wet weight of heart tissue after 5 minutes' incubation at 37° C. The automated procedure of Hochella and Weinhouse (10) was used for LDH assays using Versatol E and E-N as standards. LDH activity is expressed as International Units (I.U.) per milligram wet weight of heart tissue.

5. Statistical data. Analyses of variance in heart weights and in LDH and COx levels were made to determine any significant relationships. Data were obtained from 111 control and 108 experimental embryos with at least 15 control and 15 exposed embryos analyzed for each exposure period.

Results

Carboxyhemoglobin levels of the 17-day-old control chick embryos averaged 1.5 ± 0.8% ($\bar{x}$ ± S.D.) whereas the levels of the 17-day-old exposed chick embryos regardless of their exposure time averaged 25.4 ± 1.2% ($\bar{x}$ ± S.D.).

Hypertrophy of the heart was noticeable after 144 hours of exposure to CO. The mean heart weights (Table 1) of chick embryos exposed for 144 and 168 hours support this observation. Analysis of variance in heart weights (Table 1) shows a significant difference between the control and CO-exposed groups ($p<0.01$; $F = 4.4$) and demonstrate the relationship between length of exposure and hypertrophy of the heart.

TABLE 1

Exposure Effects of 425 ppm CO in Air on Chick Embryo Heart Weights*

	Hours of exposure							
	168	144	120	96	72	48	24	Total
Exposed	145	134	123	126	125	123	106	126
Control	118	120	105	107	117	107	112	112
Mean	131	127	114	116	121	114	109	119

*Mean heart weights in mg

Source of variation	Analysis of variance		
	Degrees of freedom	Mean Sum of squares	F test
Treatment group	1	10066	50.8**
Exposure	6	1934	9.8**
Interaction	6	875	4.4**
Within subgroup	205	198	-
Total	218	-	-

** $p<0.01$

No overall significant difference in LDH levels between the control and exposed groups is apparent (Table 2).

TABLE 2

Exposure Effects of 425 ppm CO in Air on Heart Tissue LDH Levels*

	Hours of exposure							Total
	168	144	120	96	72	48	24	
Exposed	61.5	67.2	77.5	78.2	60.1	64.8	64.1	67.6
Control	61.5	65.7	67.4	79.3	58.4	65.8	66.9	66.4
Mean	61.5	66.4	72.3	78.8	59.2	65.3	65.5	67.0

* Mean LDH activity expressed as International Units/mg wet weight of heart tissue.

Source of variation	Analysis of variance		
	Degrees of freedom	Mean sum of squares	F test
Treatment group	1	79.3	0.41 N.S.
Exposure	6	1355.4	7.00**
Interaction	6	137.2	0.71 N.S.
Within subgroup	205	193.5	-
Total	218	-	-

N.S. Not significant, $p>0.05$
** $p<0.01$

Table 3 summarizes the data on COx levels of the hearts of control and experimental embryos. The mean COx level of the hearts of the older embryos (14, 15, and 16 days) at time of exposure (72, 48, and 24 hours, respectively) was lower than the controls. This inhibition, however, diminished progressively as younger embryos were exposed for increasingly longer periods (96 to 168 hours).

TABLE 3

Exposure Effects of 425 ppm CO in Air on Heart Tissue COx Activity*

	Hours of exposure							
	168	144	120	96	72	48	24	Total
Exposed	0.154	0.109	0.135	0.126	0.087	0.099	0.097	0.115
Control	0.151	0.116	0.131	0.116	0.106	0.110	0.125	0.122
Mean	0.153	0.113	0.133	0.121	0.097	0.105	0.111	0.118

*Mean COx activity as E_{540}/mg wet weight heart tissue after 5 minutes at 37° C.

		Analysis of variance	
Source of variation	Degrees of freedom	Mean sum of squares	F test
Treatment group	1	0.003	10.0**
Exposure	6	0.011	36.7**
Interaction	6	0.002	6.6**
Within subgroup	205	0.0003	-
Total	218	-	-

**$p<0.01$.

Discussion

Exposure to 425 ppm CO does not represent a lethal level for the chick embryo. Though the COHb level is high, the viability of exposed chick embryos is well within normal limits. Previous studies (7) on chick embryos exposed to 425 ppm CO have established a direct relationship between COHb levels and age. The elevations in COHb levels from the 10th to 18th day of incubation serve to confirm the progressively increasing porosity of the tertiary membranes of the egg (2-4,11), as well as the decrease in the affinity of hemoglobin for oxygen of chick embryo blood (12,13). The 25.4 ± 1.2% ($\tilde{x}$ ± S.D.) COHb level of the 17-day-old chick embryo exposed to 425 ppm CO represents a value progressively acquired during 168 hours of exposure from the 10th through 17th day of incubation, as well as a value rapidly reached in the 24-hour exposure from the 16th to 17th day of incubation.

Cardiac enlargement appears to be related to the extended exposure and rising COHb levels and presumably represents an "adaptation" to increased tissue hypoxia. Since no equivalent hypertrophy was noted in the older embryos exposed for the shorter periods, heart enlargement and extended exposure to CO appear causally related.

Carbon monoxide competes with oxygen (O_2) for sites on the heme-protein molecule and the absolute affinities for both gases vary widely with the structure of heme. While relative affinities for CO and O_2 also vary, the affinity of COx for O_2 appears greater than for CO (14). Mean E_{540} values for COx activity of heart tissue of chick embryos exposed 24 to 72 hours to CO indicate an *in vivo* inhibition of this heme-containing enzyme (Table 3). Thus, while an age-related inhibition of COx occurs during the stated exposure times, viability is maintained most probably by the branching and cushioning compensatory mechanism postulated by Chance *et al.* (15). During longer exposure periods (96 hours and more), there has been sufficient time for compensating environmental adaptation so that COx levels of exposed chick embryos were similar to those of controls.

According to Gibson *et al.* (16), in the absence of oxygen, a partial pressure of carbon monoxide (pCO) of 0.16-0.22 mm Hg is required to half saturate the pigment. The concentration of 425 ppm in our experimental system reflects a pCO of 0.33 mm Hg which is more than sufficient to meet this requirement. In addition, Forster (17) states that it seems unlikely that tissue pCO becomes high enough to inhibit cytochrome oxidase activity under nonextreme conditions. Therefore, the effects of CO upon COx *in vivo* may be analyzed with the chick embryo, where a relative affinity of the COx heme for CO results in its *in vivo* inhibition without apparent lethal harm to the organism.

Results from Table 3 establish that had we not correlated exposure with the age of the embryo, the inhibition of COx by CO might not have been observed. Therefore, this suggests that subsequent CO-exposure studies should be age related and of 24 hours duration, in order to develop a progressive picture of in vivo CO inhibition and to relate this inhibition to the increasing permeability of the shell and progressive affinity of Hb for CO. What overall effect this short-term inhibition of a primary oxygen-reducing enzyme has on the embryo remains to be determined.

Summary

Hearts of 17-day-old chick embryos were assayed for alterations in lactic dehydrogenase and cytochrome oxidase activity following exposure to 425 ppm carbon monoxide during the previous 24 to 168 hours of incubation. The level of the heme-containing enzyme, cytochrome oxidase, was significantly less in the exposed than in the control embryos, and biologic effects of exposure and interaction were noted. There was no sigificant difference between the level of the nonheme-containing enzyme lactic dehydrogenase in the control and exposed group. Hypertrophy of the heart was noted in embryos exposed to CO for 144 and 168 hours.

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