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Cytogenetic Effects on Mouse Fetus of Acute and Chronic Transplacental In Vivo Exposure to Carbon Monoxide: Induction of Micronuclei and Sister Chromatid Exchanges

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Carbon monoxide gas is found in the atmosphere whenever society has become industrialized. In addition to the fact that Korea has become industrialized, bituminous coal is used to heat homes here, in heating systems that, if not very carefully maintained, leak this gas, resulting in a number of deaths and near deaths each winter. It has only rarely been reported by investigators that genetic damage may be done transplacentally to a human fetus by a pregnant woman's being poisoned by CO. We explored this by evaluating the damage done to the mouse fetus through an in vivo experiment, using micronucleus and sister chromatid exchange (SCE) tests. Mice were mated and pregnant ones divided into a group that received acute exposures on 3 different days, a group that received chronic exposure, and a control group. In the meantime in the control group the incidence of both micronuclei and SCE was less on the maternal side, in both the acute and chronic exposure groups, whereas the incidences of both micronuclei and SCE were more on the maternal side. However, the incidence on the fetal side was not far behind. Increasing, the dosage of carbon monoxide with gestational age increased the incidence of both micronuclei and SCE in the mother and fetus alike.

Key Words: Carbon monoxide (CO), micronuclei, sister chromatid exchange (SCE).

In our modern industrial soviety, carbon monoxide is a relatively ubiquitous gas in the atmosphere, which constitutes a growing menace to the human organisms not only because of a problem of air pollution resulting from industrial complexes and vehicular traffic, but-more insidiously-because of smoking (Longo 1977). Carbon monoxide poisoning, especially is the most serious and prevalent gas poisoning in Korea, mainly due to the public use of bituminous coal briquettes as the main domestic fuel. Earlier studies of the placetal exchange of carbon monoxide undoubtly were stimulated by an interest in carbon monoxide poisoning in pregnancy, which happened to be a controversial social issue. Fehling and Hogyes in 1877 performed the first experimental study; Hogyes concluded from his apparent failure to detect carboxyhemoglobin in fetal blood that carbon monoxide would not cross the placenta. Helpern and Strassman (1943) and Martland (1950) suspecting the passage of carbon monoxide from maternal to fetal circulation, suggested that the fetus would not die from carbon monoxide asphyxia as would the mother, but rather die from simple asphyxia, resulting from the lack of oxygen, normally supplied by the maternal blood through the placenta. This observation once led us to believe that carbon monoxide would not cross the placental barrier from mother to fetus.

On the other hand, there have been studied that investigated the possible evidence of carbon monoxide transport to the fetus, although even in these cases it was difficult to state with certainly that the high level of carbon monoxide in the fetal blood was the cause of death (Fehling 1877; Grehant and Quinquard 1883; Dreses 1891; Niclous 1901; Balthaxand and Nicoloux 1913; Williams and Smith 1935; Dörobert *et al* 1949; Seifert 1953; Curtis 1955).

Since the effects of carbon monoxide poisoning on human fetuses during pregnancy were reviewed by Breslau (1859), there have been several reports on the evidence of occurrence of fetal anomaly in the human (Ingalls *et al* 1950; Muller and Graham 1955), and in experimental animal (Lee *et al* 1974; Choi and Oh *et al* 1975).

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Contrary to these studies, Schwetz *et al* (1979) Observed the absence of teratogenic effect in mice and rabbits which inhaled carbon monoxide at a concentration of 250 ppm during the period of major organogenesis, but there were evidences found of toxicity to the fetus or embryo upon examination of the fetuses for minor alterations: a statistically significant increase in the incidence lumbar ribs and lumbar spurs among the groups exposed for 24 hours daily to the gas. To date however the genetic studies to determine the role of carbon monoxide poisoning in pregnant women in causing fetal death or congenital malformation irrespective of maternal survival have been very few. The genetic effects of carbon monoxide poisoning may be investigated in the course of extensive studies, using the micronucleus and sister chromatid exchange tests.

Considering that the most important application of the micronucleus test has been screening chemicals of unknown mutagenic potential in vivo it was felt that every possibility should be explored that promised to render the test the more practical, reliable, and sensitive. The micronucleus test is geared to detect mutagenic activities of a number of compounds during screening (Lederbur and Schmid 1973). Sister chromatid exchanges (SCEs) represent the interchange of the products of DNA replication at apparently homologous chromatid loci. SCE analysis affords an excellant opportunity for cytological detection of DNA interchange.

During the past few years, SCE analysis has been in use as a sensitive means of monitoring DNA dammage. Much of this activity has been made possible due to the development of BUdR-dye techniques for SCE detection (Latt and Schreck 1980). In vivo, the level of BUdR has been found to decrease immediately after its injection because of high metabolic activities of liver enzymes. SCE analysis in utero is known to be difficult but allows simultaneous examination of the effects of agents on the fetal and maternal cells, and it is known to be more sensitive in detecting DNA impairment than chromosomal aberrations (Perry and Evans 1975).

The purpose of our study was to explore the transplacental genetic damage done to the fetal mouse in utero by carbon monoxide, through an in vivo study on the micronucleus and sister chromatid exchange tests; and to look for the stage of pregnancy in which if damage occurs at all, this damage genetically effects the fetus.

MATERIALS AND METHODS

Two-to-four-month-old nulligravid female ICR mice were fed under identical conditions and mated with male mice of the same age and strain when their body weight reached about 30 grams. The gestational age was determined by counting the number of days after the observation of vaginal plug. The "plug day" was counted as one of the days of gestation.

The control and experimental groups of this study are outlined in the table below:

Carbon monoxide exposure

An acute exposure group was exposed for 10 minutes to concentration of 1,500, 2,500, and 3,500 ppm of carbon monoxide on the days 5, 11, and 16 of gestation, respectively.

The chronic exposure group was exposed for one hour to a concentration of 500 ppm of carbon monoxide on days 0 to 6, 7 to 13, and 14 to 20 of gestation respectively.

All exposures were conducted in a chamber under a dynamic airflow condition. The test animals were exposed to the desired concentration of carbon monoxide while the control animals were exposed to filtered room air under an identical chamber condition.

Chamber atmosphere

The carbon monoxide atmosphere was generated

Experimental groups, by various conditions

Group	Pregnant Mice N	Concentration of CO (ppm)	Duration of Exposure (min)	Day of Gestation at Exposure	Day of Gestation Killed
Control	20	0	0	–	21
Acute	64	1,500,2,500 3,500	10	5, 11, 16	21
Chronic	50	500	60	0-6, 7-13, 14-20	21

by metering carbon monoxide (Matheson 99.5% pure chemically) at a calculated rate from compressed gas cylinders into the chamber air inlets. The concentrations of 500, 1,500, 2,500, and 3,500 ppm of carbon monoxide in the chamber were calculated from the ratio of the flow rate to the rate of chamber airflow.

Observation methods

Micronucleus test

Fetal blood test: The gravid uteri were removed and placed in fetal calf serum. The fetus was removed from the uterus. The maternal membranes were dissected and the fetus was transferred to fetal calf serum and washed once. The entire fetus was cut up with scissors. The supernatant suspension was decanted.

Maternal bone marrow test: In dissecting, the femurs were left intact by cutting through the tibia and the pelvis. The bones were freed from the muscles, using gauze. The proximal end of the femur was shortened by cutting it with scissors (Heddle 1973; Matter and Grauwiler 1974; Schimid 1975). The centrifuge tube was filled with fetal calf serum up to its rim. With a Syringe to which a needle was attached, the serum from the tube was pulled into the syringe. The needle to which the syringe containing the serum was attached was inserted into the distal part of the femur and the contents of the femur were withdrawn.

The cells of the fetal blood and maternal bone marrow is collected by centrifugation at 1000 rpm for 5 minute. From the pellet smears is made on slides and air dried. Staining is performed according to May-Grünwald & Giemsa method. At least 2000 polychromatic erythrocytes is analyzed for each animal.

Sister chromatid exchange test

FUdR at the rate of 400μg/g of body weight was injected intraperitoneally once. 2h later, FUdR at the rate of 0.2μg/g of body weight and BUdR at the rate of 40μg/g of body weight was injected six times at hourly intervals. CO exposure was carried out after the initial BUdR injection. Mice were injected with 2.5μg Colcemid (GIBCO) and killed 2h later. The maternal bone marrow and fetal cell suspension was centrifuged for eight minutes at 1,000 rpm, and hypotonic treatment was carried out with 0.075M kCl, then fixed (methanol/acetic acid, 3:1), and the slides were prepared by air drying. Differential staining of chromatids was carried out, using Hoechst 33258 and Giemsa. The method of Perry and wolff (1974) and Latt (1974), as modified by Eppled *et al* (1975), was used. SCE was counted in 100 metaphases per group.

RESULTS

Micronuclei

Acute exposure group: In the control group, the incidence of micronuclei in the maternal bone marrow cells was 0.2% and in the fetal cells 0.3%, on one day in each gestational period, the percentage of the maternal and fetal micronuclei was calculated and found to have increased in direct proportion to CO exposure (1,500, 2,500, 3,500 ppm) (Table 1, Fig. 1 ($P<.01$)). When the mice were exposed to the 3 different levels of CO gas on day 5 of gestation, the incidence of micronuclei varied from 1.7 to 7.9% on the maternal side and 1.0 to 6.8% on the fetal side. Under the same conditions but on the day 11 of gestation, the incidence was 1.9 to 8.1% on the maternal side and 1.1 to 7.4% on the fetal side. On day 16, the values were 1.6 to 8.3% on the maternal side and 0.8 to 6.5% on the fetal side.

After exposure at the vaious gestational intervals, the incidence of micronuclei on the maternal side was somewhat lower than that on the fetal side. However, there was no significant statistical difference in incidence of micronuclei between the two ($P>.05$). In the exposed group, the incidence of micronuclei on

Table 1. Incidence of micronuclei in maternal and fetal cells treated with acute exposure carbon monoxide

Fetal age (days)	Conc. (ppm)	Micronucleated polychromatic erythrocytes (%)	
		Maternal bone marrow	Fetal blood
5	Control	0.2	0.3
	1,500	1.7	1.0
	2,500	4.8	3.9
	3,500	7.9	6.8
11	1,500	1.9	1.1
	2,500	5.6	4.7
	3,500	8.1	7.4
16	1,500	1.6	0.8
	2,500	5.7	3.1
	3,500	8.3	6.5

Mean values of 5 pregnant female animals cell counts were on samples comprising 2,000 polychromatic erythrocyte cells.

the fetal side was greater when exposed on day than on or 16 gestation (P>.05).

Chronic exposure group: Throughout the gestational period 500 ppm of carbon monoxide was given for one hour daily.

When compared with the control group, the chronic exposure group showed higher incidence of micronuclei on both the maternal and fetal side (Table 2, Fig. 2, P<.05). At various gestational period, the incidence of micronuclei on the fetal side was less than that on the maternal side, but there was no signifi-

Table 2. Incidence of micronuclei in Maternal and fetal cells treated with chronic exposure carbon monoxide

Fetal age (days)	Conc. (ppm)	Micronucleated polychromatic erythrocytes (%)	
		Maternal bone marrow	Fetal blood
	Control	0.2	0.3
0 - 6	500	7.4	5.7
7 - 13	500	8.1	7.2
14 - 20	500	7.5	5.1

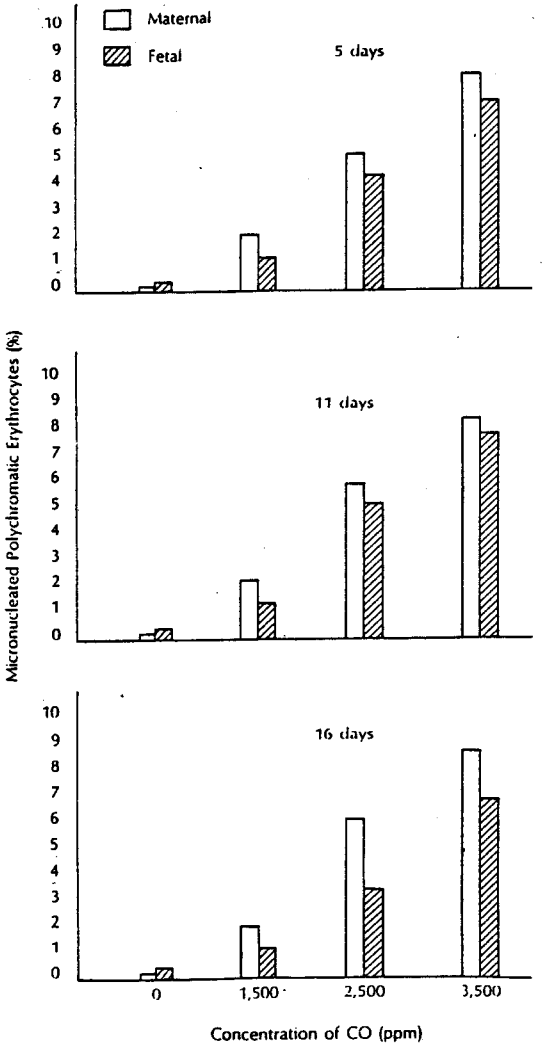

Fig. 1. Concentration – effect for acute exposure of CO by micronucleus test in maternal and fetal cells.

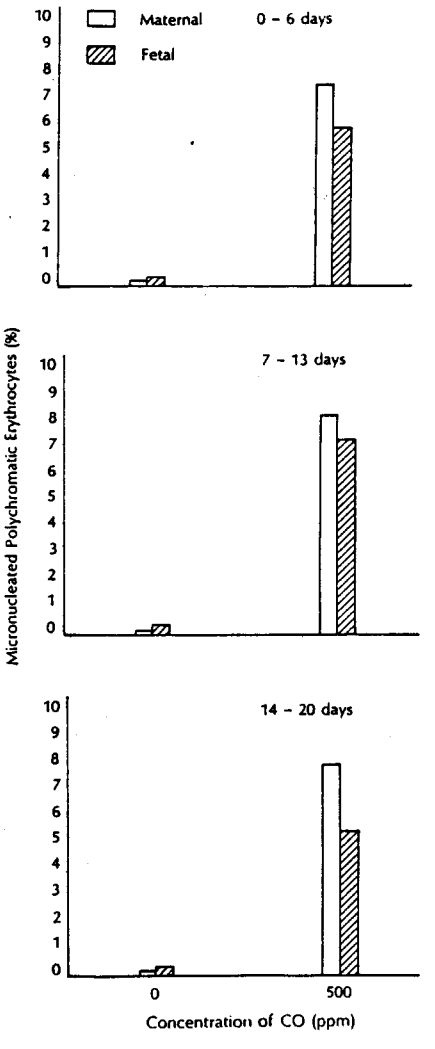

Fig. 2. Concentration – effect for chronic exposure of CO by micronucleus test in maternal and fetal cells.

Table 3. Frequencies of SCEs in maternal and fetal cells treated with acute exposure to carbon monoxide.

Fetal age (days)	Conc. (ppm)	Scored cells	Scored cells	No. of SCEs		SCEs/cell±S.E		SCEs/Chromosome	
				M	F	M	F	M	F
5	FUdR + BUdR only	100	4,000	324	332	3.2±0.27	3.3±0.36	0.08	0.08
	1,500	100	4,000	583	431	5.8±0.73	4.3±0.45	0.15	0.11
	2,500	100	4,000	1,068	882	10.7±0.67	8.8±0.79	0.27	0.22
	3,500	100	4,000	1343	1,058	13.4±1.23	10.6±0.26	0.34	0.26
11	1,500	100	4,000	634	567	6.3±0.21	5.7±0.24	0.16	0.14
	2,500	100	4,000	1,092	1043	10.9±0.38	10.4±0.52	0.27	0.26
	3,500	100	4,000	1,448	1,234	14.5±0.40	12.3±0.73	0.36	0.31
16	1,500	100	4,000	546	408	5.5±0.19	4.1±0.20	0.14	0.10
	2,500	100	4,000	1024	859	10.2±0.33	8.6±0.13	0.26	0.21
	3,500	100	4,000	1392	978	13.9±0.25	9.8±0.32	0.35	0.24

Table 4. Frequencies of SCEs in maternal and fetal cells treated with chronic exposure to carbon monoxide.

Fetal age (days)	Conc. (ppm)	Scored cells	Scored cells	No. of SCEs		SCEs/cell±S.E		SCEs/Chromosomes	
				M	F	M	F	M	F
	FUdR + BUdR only	100	4,000	337	329	3.2±0.27	3.3±0.36	0.08	0.08
0 - 6	500	100	4,000	1,084	813	10.8±0.32	8.1±0.27	0.27	0.21
7 - 13	500	100	4,000	1,123	1,038	11.2±0.14	10.4±0.19	0.28	0.26
14 - 20	500	100	4,000	1,074	776	10.7±0.28	7.8±0.36	0.27	0.19

cant statistical difference between the two (P>.05). When exposed during the days 0 to 6 of gestation, the incidence of micronuclei was 7.4% on the maternal side and 5.7% on the fetal side, when exposed during days 7 to 13, the incidence of micronucle; was 8.1% on the maternal side and 7.2% on the fetal side. When exposed during days 14 to 20, the incidence of micronuclei was 7.5% on the maternal side and 5.1% on the fetal side.

There was no change in maternal incidence of micronuclei for each gestational period. There was an increase in incidence of micronuclei on the fetal side for 7 to 13 days exposure group when compared with 0 to 6 day, or 14 to 20 days group; the difference was not statistically significant (P>.05).

Sister chromatid exchange

Acute exposure group: In the control group treated with FUdR and BUdR, the incidence of SCE was 3.2±0.27/cell on the maternal side and 3.3±0.36/cell on the fetal side. As the dosage of carbon monoxide was increase, the incidence of SCE increased in both the mother and fetus (Table 3, Fig. 3, P<.01). The incidence of SCE was 5.8±0.73 to 13.4±1.23/cell on the maternal side and 4.3±0.45 to 10.6±0.26/ cell on the fetal side when exposed the carbon monoxide on day 5 of gestation. At day 11 of gestation, the values were 6.3±0.21 to 14.5±0.40/cell on the maternal side and 5.7±0.24 to 12.3±0.73/cell on the fetal side. At day 16 of gestation, the values were 5.5±0.19 to 13.9±0.25 cell on the maternal side, and 4.1±0.20 to 9.8±0.32/cell on the fetal side. Although the SCE in-

cidence on the fetal side was somewhat lower, there was no significant difference between the mother and fetus in terms of sister chromatid exchange (P>.05).

The incidence of SCE was significantly higher at day 11 of gestation than at either day 5 or 16 of gestation. After exposure to the same dosage at various gestational periods, there was no significant in SCE (P>.05).

Chronic exposure group: At day 0 to day 6 of gestation, the incidence of SCE was 10.8±0.32/cell on the maternal side and 8.1±0.27/cell on the fetal side. From day 7 to day 13 of gestation, the incidence of SCE was 11.2±0.14/cell on the maternal side and 10.4±0.19/cell on the fetal side. From day 14 to day 20 of gestation, the incidence of SCE were 10.7±0.28 and 7.8±0.36/cell each. In the group exposed to carbon monoxide daily for an hour, the incidence of SCE was higher than the control group both in the mother and fetus (Table 4, Fig 4, P<.05).

Although the incidence on the fetal side was a little lower than on the maternal side, there was no significant difference (P>.05).

When exposure to the same levels of carbon monoxide, there was no difference in the incidence of SCE.

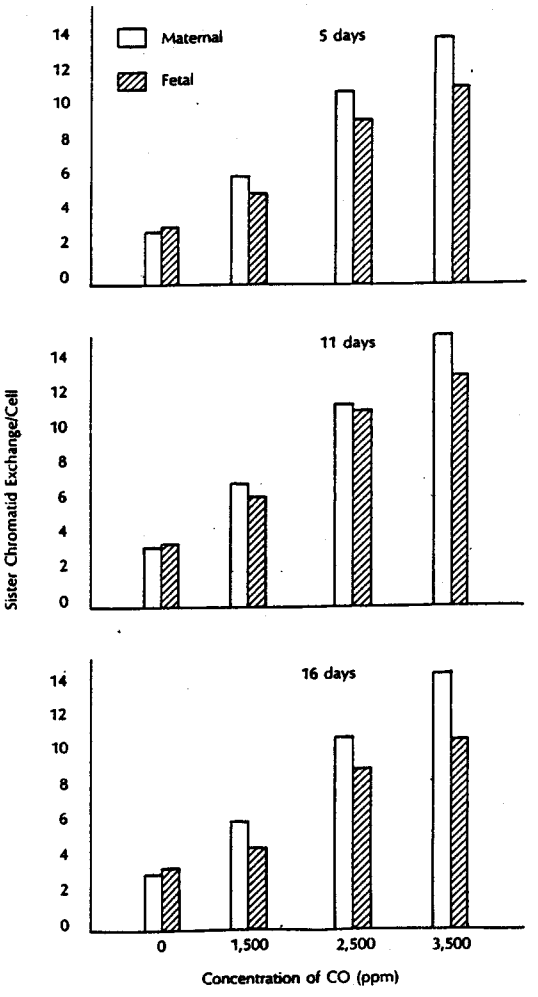

Fig. 3. Concentration - effect of exposure to CO by SCEs in maternal and fetal cells.

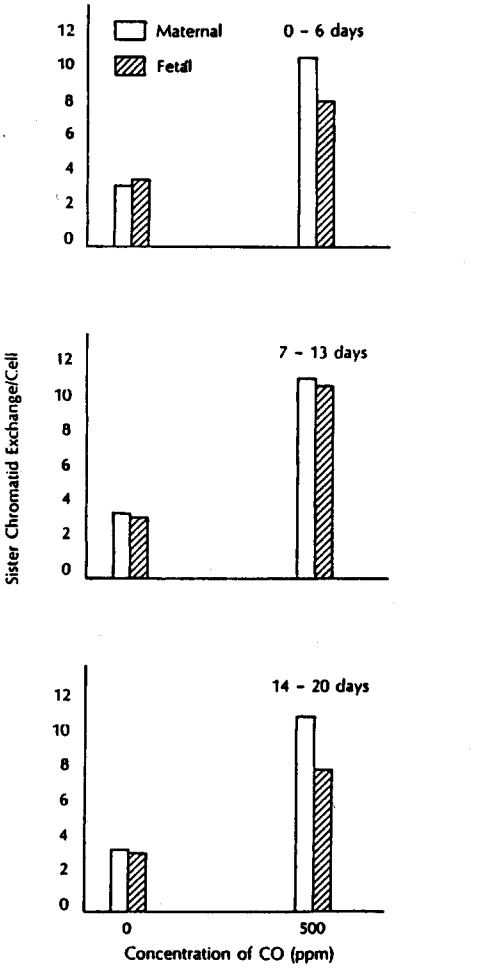

Fig. 4. Concentration - effect of chronic exposure to CO by SCEs in maternal and fetal cells.

DISCUSSION

Carbon Monoxide is a gas that is odorless, tasteless, and colorless. Chemically, carbon monoxide is a heteropolar, diatomatic molecule, which is gaseous and stable at ambient temperature and pressure. The poisonous nature of carbon monoxide results from the strength of the coordination bond formed with the iron atom of hemoglobin (Longo 1977).

Carbon monoxide can diffuse from the maternal blood stream into fetal blood (Fehling 1877; Dreser 1891; Nicloux 1901; Seifert 1952; Curtis 1955). Extensive studies on the toxic effects of carbon monoxide show that in pregnant women acute poisoning with carbon monoxide may be the cause of fetal death or congenital malformations, irrespective maternal survival.

As early as in 1857, Bernard reported that carbon monoxide was hazardous to human health. In 1959, Lilienthal observed that the oxygen supplying hemoglobin in human tissue combines with CO to form HbCO, causing depletion of oxygen. Breslau in 1859 made the first clinical observation of the effects of acute illuminating intoxication of the fetus which died in the eighth month of gestation. Wells (1933) observed that when pregnant rats were exposed to 0.59% carbon monoxide for 5 to 8 minutes daily, no notable effects were seen. When, however, the exposure was increased to 1.5%, spontaneous abortions occured. Schwetz (1979) exposed pregnant mice daily to 250 ppm of carbon monoxide for from 7 to 24 hours. This resulted in a statistically higher incidence of lumbar spurs but no significant fetal malformations. He concluded that carbon monoxide was thus toxicogenic but not mutagenic. Although our study in different from the studies previously conducted, ours suggests that the fetal and maternal incidence of micronuclei and the frequency of SCE would increase according to the concentration of carbon monixide supporting the hypothesis that carbon monoxide is toxicogenic to the maternal and fetal body.

Two theories on the mechanisms and effects of CO toxicity in pregnancy have been conflicting: one is that simple hypoxia due to CO secondarily affects the fetus (Helpern and Strassmann 1943; Martland and Martland 1950) and the other is that CO in maternal blood crosses the placenta, causing fetal CO intoxication, leading to fetal death (Curtis *et al* 1955; Friberg *et al* 1959). From the animal studies, the theory that CO crosses the placenta and forms HbCO in the fetal blood has been strongly supported. Classically, the mechanism of transplacental carbon monoxide exchange had been assumed to be the one in which the gas passively diffused through the lung, placenta, and other tissues moving in the direction of lower partial pressure gradient.

According to our study, the increase of micronuclei and SCE in both the mother and fetus in response to the increase of CO concentration indicate that CO crosses the placenta and affects the fetus. There was no significant different between the mother and fetus, because the placenta did not serve as a barrier, protecting the fetus against CO. Wilson (1953) reported 2.6% teratogenic incidence in rats, caused by CO toxicity during the initial period of formation and no damage during the late period of organ formatioin and the early period of tissue formation. This implied that the effects of CO toxicity vary with the stage of fetal development. In our study, the acute exposure group, on day 11 of gestation, showed a higher rate of appearance of micronuclei and SCE compared with that on days 5 and 16 of gestation, indicating that the most carbon monoxide crossed the placenta during the period the fetus was most sensitive to hypoxia. In the chronic exposure group, however, the 7-to-13-day exposure group showed more increase in the incidence of micronuclei and SCE than the 0-to-6-day and the 14-to-20-day exposure groups. They did not show a significant difference according to the gestational period.

Since the concentration of carboxyhemoglobin in fetal blood varies as a function of maternal carboxyhemoglobin concentration, the rate of fetal CO production, the diffusing capacity of the placenta for CO, the relative affinity of hemoglobin for CO, as compared with O_2, and the affinity of blood for O_2 and for CO (Longo 1979) would also vary in different species. Rats are less sensitive to CO, and the blood level of HbCO in their case, has returned to normal within 24 hours. In our study with mice, no attempt was made to observe the HbCO level because of the lack of facilities. There remains a need for more experiments to be done on more subjects, testing with various concentrations.

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