percent, and this value was used as the extra cellular space for correcting the data. This value was not significantly different at external K concentrations of 2.5, 100, and 400 m*M*, and was constant over an 8-hour period at the two higher concentrations. At 2.5 m*M* K the sucrose space rose gradually from 13 to 20 percent after 8 hours of incubation in sucrose. The inulin space of muscles in control solutions was 11 percent.

17. The time course of changes in the K, Na, Cl, and H_2O for up to 8 hours was determined in each of the solutions. It was found that steady levels were achieved in less than 1 hour after transferring the cells to the final solution. The steady levels were maintained for 8 hours in all solutions studied except for the two with the highest K_{ex}. In these solutions (K_{ex} = 450 and 500 m*M*), the cells gradually gained Na and H_2O above the physiological levels at 2 hours and beyond. Our results for Cl uptake differed somewhat from those of Adrian (*21*), who found a continuous rise in cell Cl from 1 to 5 hours in 100 m*M* KCl. The discrepancy may be due to a species difference or to slight differences in Ringer solutions.
18. D. M. Needham, *Machina Carnis* (Cambridge Univ. Press, Cambridge, England, 1971), p. 194.
19. A double-reciprocal plot of the data ($1/K_{cell}$ against $1/K_{ex}$) is nonlinear. If the points at $K_{ex} \geq 150$ m*M* are used, the data fall approximately on a straight line, giving an extrapolated saturation value of 2500 μmole per gram of tissue. This is an order of magnitude larger than the number of fixed negative charges in the cell.
20. In solutions with high K_{ex} the muscles lost a small amount of water, the water content falling from 80 percent in control solution to 77 percent. As discussed by Adrian (*21*), this shrinking may reflect the inequality of the ϵ and η terms.
21. R. H. Adrian, *J. Physiol. (London)* **151**, 154 (1960).
22. The initial phases of this study were carried out at the Department of Molecular Biology, Pennsylvania Hospital, Philadelphia. Supported by PHS grant HL-07762.

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Carbon Monoxide: Effects on Oxygenation of the Fetus in utero

Abstract. *The partial pressure of oxygen in fetal blood decreases in proportion to the carboxyhemoglobin concentrations in fetal and maternal blood. Because fetal oxygen tensions normally equal 20 to 30 percent of the values for adults, this reduction can result in severe hypoxia of vital tissues. Decreases in oxygen tension may be a factor in the lower birth weights of infants born to women who smoke or are exposed to severe air pollution.*

Bernard (*1*) first showed that the physiologic effects of CO result from decreased capacity of the blood to transport O_2. Several workers (*2, 3*) have predicted that increased blood carboxyhemoglobin (HbCO) concentrations should result in decreased O_2 partial pressures in outflowing (venous) blood from a particular tissue bed. The question remains, however, to what extent these changes occur in vivo in the presence of possible compensatory changes in blood flow. An additional question concerns the effects of elevated blood HbCO concentrations on blood O_2 levels in the fetus, in which the O_2 partial pressure in arterial blood is normally only 20 to 30 torr, compared with about 100 torr in the adult. The work reported here was done to explore these questions.

I implanted small Tygon catheters in a maternal artery and a branch of the uterine vein of pregnant sheep. In the fetus, catheters were placed in a pedal artery and passed into the descending aorta, and in a pedal vein and passed into the inferior vena cava below the ductus venosus. Four or more days after recovery, I exposed the ewes to CO concentrations of 30, 50, or 100 parts per million (ppm) for 36 to 48 hours in an effort to achieve equilibrium between maternal and fetal partial pressures of carbon monoxide. Nine to 11 experiments were performed with six or seven different animals at each CO concentration. Experiments were not performed more frequently than at weekly intervals in animals that underwent repeated study. I measured oxygen partial pressures and HbCO concentrations repeatedly during CO uptake and equilibrium. Inspired CO was monitored with a CO analyzer (Ecolyzer, Energetics Science, Inc., Elmsford, N.Y.). Blood HbCO was determined spectrophotometrically by using an instrument (CO-Oximeter, model 182, Instrumentation Laboratory, Lexington, Mass.) that was recalibrated daily to maintain an accuracy of ± 1 percent in the HbCO range from 1 to 30 percent saturation. Blood partial pressures of O_2 and CO_2 and blood *p*H were determined by using appropriate microelectrodes (Radiometer model BMS 3, London Co., Westlake, Ohio).

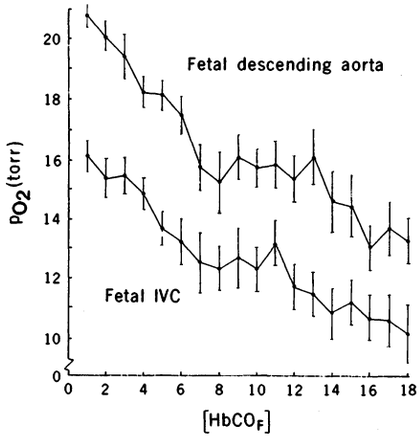

Fig. 1. Oxygen partial pressures in blood of the fetal descending aorta and the inferior vena cava (*IVC*) as a function of fetal HbCO concentrations during quasi-steady-state conditions of maternal-to-fetal CO exchange. Fetal inferior vena cava O_2 tension varies as a function of both maternal and fetal HbCO concentrations. The O_2 tension of fetal arterial blood is chiefly a function of maternal HbCO concentration. However, during steady-state conditions it is also related to the fetal HbCO concentration. Each point represents the mean ± standard error of the mean (vertical bars) of 6 to 20 determinations at each HbCO concentration. The indicated HbCO concentrations are actually ± 0.5.

Control values of maternal and fetal HbCO concentrations determined before exposure to CO, were, respectively, $[HbCO_M] = 1.1 \pm 0.2$ percent and $[HbCO_F] = 1.8 \pm 0.3$ percent (mean ± standard error of the mean). During maternal-to-fetal CO exchange after exposure, O_2 partial pressures decreased in the maternal uterine vein and in the fetal arterial and venous blood. The O_2 tension of uterine venous blood decreased from a control value of about 43 torr at a maternal HbCO concentration of 1 percent to about 39 torr at 10 percent. The least-squares regression equation for this relation was $pO_2 = 43.1 - 0.5[HbCO_M]$ (correlation coefficient $r = -0.96$), where pO_2 is the oxygen partial pressure.

Figure 1 shows that, under these quasi-steady-state conditions, the O_2 partial pressure in the fetal descending aorta decreased to 16 torr at a fetal HbCO concentration of 10 percent, from a control value of about 20.5 torr (*4*). The regression equation for this relation was $pO_2 = 20.1 - 0.4[HbCO_F]$ ($r = -0.94$). These O_2 tensions represent the mean values of 6 to 20 determinations at each HbCO concentration. An HbCO concentration of 10 percent actually represents the values that were greater than 9.5 percent but less than 10.5 percent, rather than exactly 10.0 percent. Figure 1 also shows the relation of O_2 tension in the fetal inferior vena cava, below the ductus venosus, to the fetal HbCO concentration. At a fetal HbCO concentration of 10 percent, the O_2 tension in the inferior vena cava decreased from a control value of 16 torr to about 13 torr. The regression equation for this relation was $pO_2 = 15.8 - 0.3[HbCO_F]$ ($r = -0.96$).

The O_2 tension in fetal arterial blood reflects the adequacy of placental exchange. Strictly speaking, it is a function of the O_2 tensions in the maternal and fetal placental end capillaries, which in turn are related to the maternal HbCO concentration. The O_2 partial pressure in fetal venous blood reflects the adequacy of tissue oxygenation. It decreases as a result of increased fetal and maternal HbCO concentrations (which result in

decreased maternal and fetal placental end-capillary O_2 tensions) (*4*).

Since the O_2 partial pressures were obtained when maternal and fetal HbCO concentrations were in quasi-steady-state equilibrium, a relation between fetal O_2 tensions and both maternal and fetal HbCO concentrations would be expected. (It should be noted that, under steady-state conditions, the ratio $[HbCO_F]/[HbCO_M]$ is about 1.6 in sheep and 1.1 in humans.) The regression equations for fetal O_2 tension as a function of maternal HbCO concentration were: descending aorta, $pO_2 = 20.8 - 0.6[HbCO_M]$ ($r = -0.97$); and inferior vena cava, $pO_2 = 16.1 - 0.5[HbCO_M]$ ($r = -0.94$). These partial pressures of O_2 in sheep are, of course, not identical with the values that would be anticipated in humans, because of differences in O_2 affinities and capacities of maternal and fetal blood between the species. However, I estimate these differences to equal no more than 2 or 3 torr.

Figure 2 shows the theoretical basis for understanding the effect of CO on blood O_2 in humans. Fetal blood has a greater O_2 affinity than maternal blood, hence the oxyhemoglobin saturation curve for fetal blood lies to the left of the adult curve. In addition, human fetal blood contains more hemoglobin than maternal blood (16.3 compared to 12 g per 100 ml) and therefore possesses a greater capacity to transport O_2. Under normal circumstances, the O_2 tension of maternal arterial blood equals about 98 torr and its O_2 content is about 16.1 ml per 100 ml of blood (point A_{M1} in Fig. 2). In the placenta, about 5 ml of O_2 is extracted per 100 ml of blood, which produces a mixed venous O_2 tension in the uterus of about 34 torr (point V_{M1}). Increasing HbCO in the maternal blood decreases the O_2 capacity and shifts the oxyhemoglobin saturation curve to the left. Although the arterial O_2 tension remains essentially normal (*5*), the content decreases to 14.5 ml per 100 ml (point A_{M2}). With the same placental O_2 transfer, the venous O_2 tension would equal 27 torr (point V_{M2}), about 7 torr less than normal.

In the fetus, the O_2 partial pressure in the descending aorta normally equals about 20 torr, and the O_2 content is about 12 ml per 100 ml (point A_{F1} in Fig. 2). With an O_2 consumption of 5 ml/100 ml, the O_2 tension in the inferior vena cava would be 16 torr (point V_{F1}). Elevated fetal HbCO concentration of 10 percent reduces both arterial (point A_{F2}) and venous (point V_{F2}) O_2 tensions. With normal fetal O_2 consumption, the venous O_2 content would be 5 ml per 100 ml of blood less than the arterial content, which would produce a venous O_2 tension of about 11 torr, 5 torr less than normal. It should not be thought that fetal O_2 tensions decrease only as both fetal and maternal HbCO concentrations increase. They decrease even under non-steady-state conditions, when maternal HbCO increases significantly but fetal HbCO remains low. Such conditions are present during measurements of the placental CO diffusing capacity (*6*) and in acute CO poisoning in the pregnant woman.

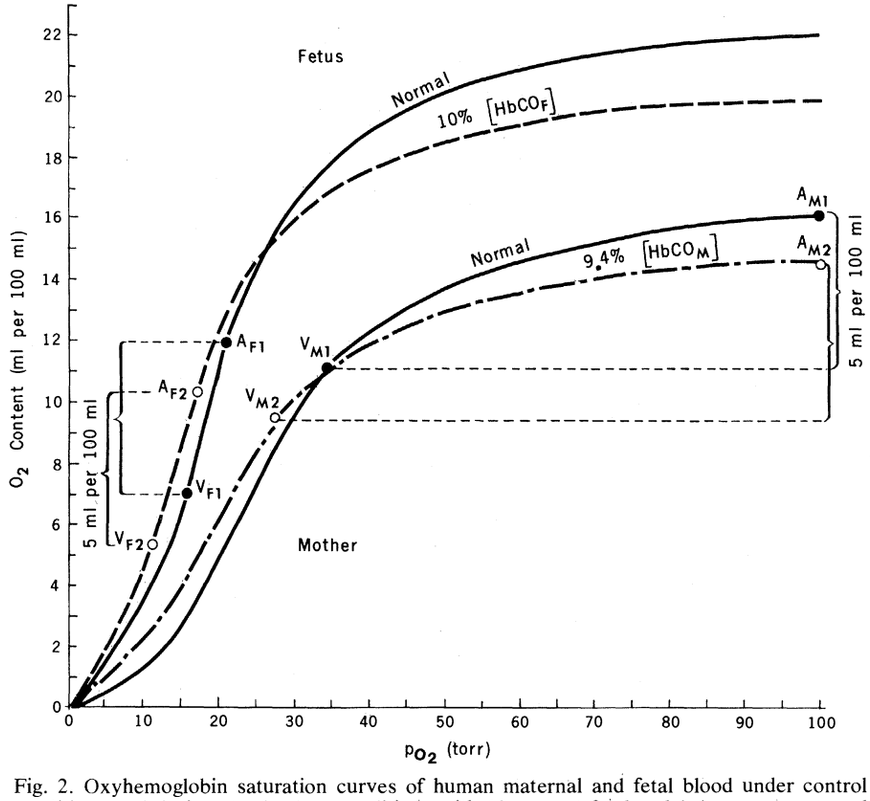

Fig. 2. Oxyhemoglobin saturation curves of human maternal and fetal blood under control conditions and during steady-state conditions with 10 percent fetal and 9.4 percent maternal HbCO concentrations. The maternal and fetal hemoglobins contents were assumed to equal 12 and 16.3 g per 100 ml of blood, respectively. A normal O_2 consumption of 5 ml per 100 ml of blood was assumed for both the uterus and its contents and the fetus (see text for details).

About 57 percent of the ovine fetuses died when fetal HbCO concentrations remained greater than 15 percent for 30 minutes or longer (*4*). At a CO concentration of 100 ppm, 5 of 11 fetuses died, and at 300 ppm, 3 of 3 died. (The studies at 300 ppm continued for only 2.5 to 3 hours, rather than until equilibrium was achieved, and are not otherwise included in this report.) Presumably these deaths resulted from hypoxia of vital tissues. Two major factors probably account for this. First, in the adult, elevation of HbCO concentration to 15 to 20 percent results in a decrease in venous O_2 tension of 6 to 10 torr. While this is a substantial decrease, the resulting O_2 partial pressures probably lie well above critical values for maintenance of O_2 delivery to the tissues and for aerobic metabolism (*7*). In the fetus, where normal arterial and venous O_2 tensions are probably close to the critical levels, a substantial decrease in O_2 tension can result in tissue hypoxia or anoxia. Furthermore, adult animals subjected to CO hypoxia show increases in cardiac output (*8*) and presumably in tissue blood flow. Apparently the fetus cannot make such adjustments, because the measured decreases in fetal O_2 tensions were about what would be expected in the absence of increased tissue blood flow. In addition, the fetus probably cannot significantly increase its cardiac output which normally equals about two to three times that of the adult per unit weight (*9*). Thus, the fetus probably normally operates near the peak of its cardiac function curve.

The observations reported here suggest that significant increases in maternal and fetal HbCO concentrations, as in mothers who smoke or are exposed to severe air pollution, can significantly reduce O_2 delivery to fetal tissues. It has been reported that a mother who smokes is nearly twice as likely to deliver a low-birth-weight infant as a nonsmoking mother (*10, 11*), and that birth weights of infants whose mothers smoke average 150 to 325 g less than those of paired controls (*11–13*). The incidence of stillbirths

and deaths of newborn children is also higher for mothers who smoke, the rates being approximately dose-related (*11*, *14*). There are undoubtedly several mechanisms for these effects since tobacco smoke contains many chemicals. Because HbCO concentrations of 5 to 10 percent are common in persons who smoke one to two packs of cigarettes per day, it is conceivable that CO-induced hypoxia may be an important factor. For instance, in a pregnant woman smoking one pack of cigarettes a day, with an HbCO concentration of about 4.8 percent, fetal O_2 tension would decrease 2 to 3 torr. In a woman smoking two packs a day, with an HbCO concentration of about 9 percent, fetal O_2 tension would decrease 4 to 6 torr. In a woman exposed to ambient CO concentrations of 30 ppm for prolonged periods, as in industrial exposure or severe air pollution, the increases in maternal and fetal HbCO concentrations and decreases in fetal O_2 tensions would be equivalent to those expected if the women smoked a pack of cigarettes per day. Thus, fetuses of pregnant women in these environments may be exposed to CO concentrations that are not innocuous.

These results raise numerous questions regarding the biologic effects of relatively low CO concentrations on the developing embryo and fetus. For instance, what are the physiologic effects of these decreases in blood O_2 tension on availability of O_2 to the fetal brain, heart, and other vital tissues? Are cells of the developing embryo or fetus more or less sensitive to the effects of CO than those of adults? To what extent does CO interference with fetal oxygenation also result in problems such as mental retardation, cerebral palsy, and perhaps subclinical neurologic, intellectual, or behavioral deficits? Is there a threshold level above which adverse effects are noted? If so, what are the maximum allowable CO exposures for pregnant women and their fetuses?

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Thyroxine-Induced Activation of Hypothalamo-Hypophysial Axis in Neotenic Salamander Larvae

Abstract. *Thyroxine injected into the hypothalamus of neotenic* Ambystoma tigrinum *induces metamorphosis by activating hypothalamo-hypophysial stimulatory control of thyroid activity, thereby removing the hypothalamic block to metamorphosis.*

We have examined the role of thyroxine (T_4) on hypothalamic function in tiger salamander larvae with respect to hypothalamic maturation and control over metamorphosis. A number of reports have dealt with interactions among thyroid hormones, thyrotropin [thyroid-stimulating hormone (TSH)], prolactin (PRL), gonadotropins, corticotropin, melatonin, gonadal steroids, and corticosteroids in neotenic populations of tiger salamanders, *Ambystoma tigrinum*, in Colorado (*1–3*). These animals normally become sexually mature and breed without first undergoing metamorphosis to adult terrestrial body form and are termed neotenes. This neotenic condition cannot be explained by reduced sensitivity of peripheral tissues to thyroid hormones or of the thyroid gland to TSH since both neotenes and immature larvae from populations that normally undergo metamorphosis before sexual maturation do not differ in sensitivities to these hormones (*2*, *4*). Neotenes occasionally undergo spontaneous metamorphosis, especially when brought into the laboratory in the spring and fall.

We examined the influence on metamorphosis of a small amount of T_4 placed in the region of the hypothalamus. Neotenes were anesthetized lightly with urethane, and a small hole was drilled in the roof of the mouth posterior to the optic chiasma but anterior to the pituitary. We injected T_4 into the region of the hypothalamus in a single dose of 2.0, 0.2, or 0.02 μg in 5 μl of saline buffer (*5*). The hole in the skull was occluded with dental cement. Placement of the injected dose was aided by the use of a stereotaxic device (*6*). A high incidence of metamorphosis was observed in all but the group receiving the lowest dose (Table 1). Animals receiving saline buffer intrahypothalamically (i.h.) did not metamorphose. This experiment was repeated and almost identical results were obtained. Neotenes or immature larvae receiving a single intraperitoneal (i.p.) dose of T_4 do not metamorphose. Injection of the largest dose of T_4 (2.0 μg) into the olfactory region of the brain induced metamorphosis in only three of eight neotenes.

In a second series of experiments, we measured the uptake of radioiodide by the thyroid of neotenes following a single i.h. injection of 2.0 μg of T_4, compared to effects of daily injections of 0.2 μg of T_4 administered i.p. for 10 days (Table 2). Daily injections of 0.2 μg of T_4 had been shown previously to induce metamor-

Table 1. Effectiveness of single i.h. injections of T_4 in inducing metamorphosis in immature and neotenic larvae of *Ambystoma tigrinum*. Larvae were maintained at 15°C on a photoperiod of 12 hours light and 12 hours darkness. The injection vehicle was 5 μl of saline buffer, *p*H 10.4 (*2*, *4*). Mean body weight values are ± standard errors.

Group	*N*	Initial mean body weight (g)	T_4 dose (μg)	Proportion induced
		Neotenes		
1	8	64.1 ± 4.13	2.0	8/8
2	8	58.2 ± 1.81	0.2	7/8
3	8	58.7 ± 2.95	0.02	3/8
4	8	59.0 ± 2.16	0	0/8
		Immature larvae		
5	9	6.0 ± 0.61	2.0	9/9
6	9	6.0 ± 0.61	0.2	0/9
7	9	6.8 ± 0.62	0	0/9