Also contributing to the successful demonstration of cross-resistance may have been the massive antigenic stimulation provided by the intraamniotic inoculation of 50 ml of stool filtrate rich in bovine rotavirus particles. A low level of virulence of the HRV-D for newborn calves may also have favored the demonstration of cross-protection. Finally, the immune response of the fetal calf may be more effective against rotavirus antigens than that of the human infant.

The ability of human rotavirus (type 2) to cross-protect against a different strain of calf rotavirus from that used in our study was examined recently in a study conducted in the United Kingdom (*18*). One of three calves infected at birth with human rotavirus was protected against disease on later challenge with bovine rotavirus, whereas two were not protected. In contrast, in an Australian study, infection of gnotobiotic piglets with human rotavirus (type 2) conferred protection against subsequent challenge with porcine rotavirus (*19*).

The ability of bovine rotavirus to protect against type 2 human rotavirus in newborn calves suggests that the bovine virus should be evaluated further for its potential usefulness in vaccination. When further studies in animals have demonstrated the safety and feasibility of this approach, a suitable bovine rotavirus should be tested in human adults who possess immunoglobulin A intestinal antibody to rotavirus and subsequently in adults who lack such antibody. Since the human virus infects newborn monkeys, calves, and piglets, the bovine virus may be found capable of infecting man.

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Eye Malformations in Rats: Induction by Prenatal Exposure to Nickel Carbonyl

Abstract. *Exposure of pregnant rats to inhalation of nickel carbonyl on days 7 or 8 of gestation frequently causes the progeny to develop ocular anomalies, including anophthalmia and microphthalmia. The incidence of extraocular anomalies is very low. The specificity of nickel carbonyl for induction of ocular anomalies in rats appears to be unique among known teratogenic agents.*

Nickel carbonyl, $Ni(CO)_4$, is a volatile liquid (boiling point, 43°C) that is an intermediate product in the Mond process for nickel refining. The compound is also used for vapor plating of nickel in the semiconductor industry and as a catalyst in the plastics, rubber, and petroleum industries (*1*). For many years, $Ni(CO)_4$ has been known to be extremely toxic for man and experimental animals, and it has been shown to be carcinogenic for rats (*1*). We have investigated the possibility that $Ni(CO)_4$ might also be teratogenic. We report here that exposure of pregnant rats to inhalation of $Ni(CO)_4$ vapor on days 7 or 8 of gestation causes a high incidence of ocular malformations in the progeny, including absence of eyes (anophthalmia) and abnormally small eyes (microphthalmia). Numerous teratogenic agents have been reported to induce in rodents a spectrum of congenital malformations including ocular anomalies (*2*); however, our findings are striking for four reasons. First, $Ni(CO)_4$ appears to be a teratogen that almost exclusively affects the eyes in rats, with only rare occurrence of other anomalies. Sec-

Table 1. Embryotoxicity and teratogenicity of $Ni(CO)_4$ in rats that were observed for 16 weeks after birth. Dams were exposed to 0.30 mg of $Ni(CO)_4$ per liter per 15 minutes, or to ambient air (controls), on day 7 of gestation and were allowed to deliver and nurse their pups. To compare the data we used Fisher's exact test, Student's t-test, or χ^2 test, as appropriate.

Observations	Controls	$Ni(CO)_4$-exposed
Length of gestation (days)*	21.8 ± 0.5	21.8 ± 0.4
Litters with malformed live pups	0 out of 8	6 out of 9†
Live pups per litter*	10.9 ± 2.5	8.7 ± 2.6‡
Live pups with malformations		
Total	0 out of 87	22 out of 78‡
With bilateral anophthalmia	0	4
With unilateral anophthalmia	0	7
With bilateral microphthalmia	0	5
With unilateral microphthalmia	0	4
With anophthalmia and microphthalmia	0	2
With other anomalies	0	0
*Weight of pups (g)**		
Males at 4 weeks	50 ± 8	41 ± 6‡
Males at 16 weeks	267 ± 24	232 ± 15‡
Females at 4 weeks	49 ± 6	41 ± 1‡
Females at 16 weeks	167 ± 11	155 ± 18‡

* Mean ± standard deviation. † $P < .01$. ‡ $P < .001$.

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ond, ocular teratogenicity of $Ni(CO)_4$ occurs at an unusually early time in gestation, prior to formation of the primary optic vesicle as an outgrowth of the forebrain (*3*). Third, demonstration that $Ni(CO)_4$ is both carcinogenic and teratogenic furnishes additional experimental support for the hypothetical association between carcinogenicity and teratogenicity of chemical compounds (*4*). Fourth, recent changes in employment practices have increased the number of female workers in nickel refineries and chemical plants, and have thereby increased the risk of accidental exposure of women to inhalation of $Ni(CO)_4$ during pregnancy. For these reasons, the induction of anophthalmia and microphthalmia in rats by prenatal exposure to $Ni(CO)_4$ has important implications in regard to the understanding of ocular embryogenesis and the recognition of a previously unsuspected teratogenic hazard in industry.

Adult female rats of the Fischer-344 strain were caged with male breeders on successive nights until copulation was confirmed by the presence of sperm in a vaginal smear (day 0 of gestation). Rats were placed individually in an all-glass exposure chamber for a single 15-minute exposure on day 7, 8, or 9 of gestation to inhalation of $Ni(CO)_4$ vapor, as previously described (*5*). Atmospheric concentrations of $Ni(CO)_4$ within the exposure chamber were 0.08, 0.16, or 0.30 mg of $Ni(CO)_4$ per liter of air, as measured by gas chromatography (*6, 7*). The amount of $Ni(CO)_4$ that was lethal for 50 percent (LD_{50}) of nonpregnant rats was 0.58 mg of $Ni(CO)_4$ per liter per 15 minutes (standard error, ± 0.09) (*7*). Pregnant control rats were placed in the chamber for 15 minutes of sham exposure (ambient air), or for 15 minutes of exposure to inhalation of carbon monoxide in air (0.5 percent CO, by volume) (*8*).

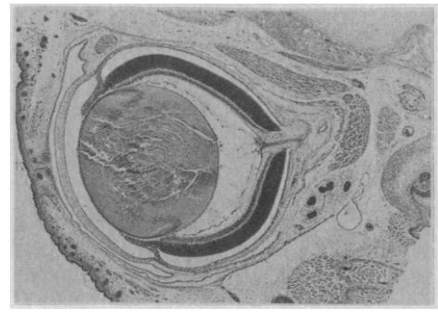

Fig. 1. Histological section of a normal eye of a rat fetus that was exposed to $Ni(CO)_4$ on day 9 of gestation and killed on day 20 (hematoxylin and eosin, ×33).

In one experiment, pregnant rats were allowed to deliver and nurse their pups until 4 weeks of age. These progeny were observed for more than 16 weeks after birth. In other experiments, pregnant rats were anesthetized with ethyl ether on day 20 of gestation, and the fetuses were delivered by cesarean section. These fetuses were examined for congenital malformations, including cranial, visceral, and skeletal anomalies, as described (*9*). Histopathologic sections were prepared from selected $Ni(CO)_4$-exposed fetuses with ocular anomalies. Coronal sections of the head were cut, and the areas that normally contain the lids, conjunctivae, and globes were examined in detail. Sections containing the optic nerves, chiasmata, optic tracts, radiations, and suprachiasmatic nuclei of the hypothalamus were also examined. Sections from $Ni(CO)_4$-exposed fetuses were compared to corresponding sections from control fetuses of the same age.

In the experiments shown in Table 1, ocular malformations were present in 22 out of 78 (28 percent) of the progeny of the $Ni(CO)_4$-treated dams. Since cross-fostering experiments were not performed, it is possible that the reduced body weights of the $Ni(CO)_4$-exposed pups were due to problems in lactation of the $Ni(CO)_4$-exposed dams. In the experiments shown in Table 2, 64 fetuses with ocular anomalies were found among 433 fetuses whose dams were exposed to $Ni(CO)_4$ on days 7 or 8 of gestation. Only two extraocular anomalies were detected: one instance of aortic transposition in an otherwise normal fetus, and one instance of hydronephrosis in a fetus which also had unilateral anophthalmia. In fetuses that were exposed to $Ni(CO)_4$ on day 7 of gestation, the ratio of anophthalmic eyes to microphthalmic eyes (33:27) was significantly higher than the ratio of 2:19 in fetuses that were exposed on day 8 of gestation ($P < .0005$ by χ^2 test) (*10*). In litters of dams exposed to $Ni(CO)_4$ on days 7 or 8 of gestation, dose-response relationships were observed between the atmospheric concentrations of $Ni(CO)_4$ and the incidences of fetal malformations. No anomalies occurred in fetuses whose dams were ex-

Table 2. Teratogenicity of $Ni(CO)_4$ in rats that were delivered by cesarean section on day 20 of gestation. Exposure of $Ni(CO)_4$ is expressed in milligrams per liter per 15 minutes. Day of exposure indicates the day during gestation on which dams were exposed to $Ni(CO)_4$.

Observations	Control groups		Groups exposed to $Ni(CO)_4$				
	A	B	C	D	E	F	G
Exposure	Sham	CO	0.16	0.30	0.08	0.16	0.16
Day of exposure	8	7	7	7	8	8	9
Dams that lived to day 20	12 out of 12	22 out of 22	14 out of 14	10 out of 19†	16 out of 16	13 out of 15	13 out of 13
Corpora lutea per dam*	10.4 ± 1.6	11.0 ± 1.0	11.5 ± 0.9	11.0 ± 1.4	10.1 ± 2.2	10.8 ± 1.3	9.6 ± 3.8
Live fetuses per litter*	9.2 ± 2.1	8.3 ± 2.6	8.1 ± 2.6	9.1 ± 1.6	7.6 ± 3.6	8.3 ± 2.6	7.4 ± 4.8
Live fetuses per total conceptuses	110 out of 114	187 out of 215†	113 out of 135†	91 out of 100‡	121 out of 134‡	108 out of 120‡	96 out of 112‡
Weight of live fetuses (g)*	3.4 ± 0.2	3.1 ± 0.7	3.0 ± 0.3†	3.0 ± 0.4†	3.3 ± 0.5	3.1 ± 0.3†	3.2 ± 0.3†
Litters with malformed fetuses	0 out of 12	0 out of 22	9 out of 14§	9 out of 10§	2 out of 16	9 out of 13§	0 out of 13
			Fetuses with malformations				
Total	0	0	15§	29§	2	19§	0
Bilateral anophthalmia	0	0	3	6	0	0	0
Unilateral anophthalmia	0	0	4	4	1	1	0
Bilateral microphthalmia	0	0	0	1	0	7	0
Unilateral microphthalmia	0	0	6	12	1	11	0
Anophthalmia and microphthalmia	0	0	1	6	0	0	0
Other anomalies	0	0	1‖	1¶	0	0	0

* Mean ± standard deviation. † $P < .01$. ‡ $P < .05$. § $P < .001$. ‖ Aortic transposition. ¶ Hydronephrosis (the fetus with hydronephrosis also had unilateral anophthalmia).

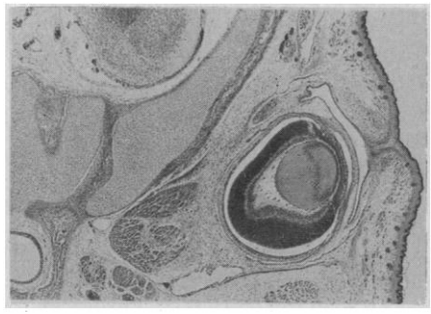

Fig. 2. Histologic section of a microphthalmic eye of a rat fetus that was exposed to $Ni(CO)_4$ on day 8 of gestation and killed on day 20 (hematoxylin and eosin, ×33).

posed to $Ni(CO)_4$ on day 9 of gestation, or in fetuses whose dams were given either sham exposures on day 8, or exposures to inhalation of CO on day 7.

Histopathological examinations of the $Ni(CO)_4$-exposed fetuses showed that eye development ranged from being indistinguishable from the controls (Fig. 1), through various degrees of microphthalmia (Fig. 2), to complete anophthalmia (Fig. 3). There was often no correspondence between the degree of development of the two eyes in a single fetus. In instances of complete anophthalmia, no ocular or related structures could be found, except the lid fissure and the conjunctival space. In instances of extreme microphthalmia, a tiny, rudimentary lens was the only demonstrable ocular structure. Such tiny lenses were often composed entirely of nucleated cells, but occasionally such lenses contained an inner circle of anuclear cells surrounded by nucleated cells. Residual lens vesicles were sometimes observed. In instances of moderate microphthalmia, there was delayed maturation and differentiation of structures in the anterior segment of the globe. Marked variations in lens appearance were seen and frequently there was evidence of cataract formation. The retinas generally appeared similar in structure to the retinas of eyes of control animals, but the retinas of microphthalmic eyes were usually thickened, folded, or redundant. In a few instances, there was reduced thickness of the neuroblastic layer; necrosis or pyknosis of retinal cells, and attenuation of the optic nerve. In some eyes, the developing optic nerve was misdirected toward the vitreous cavity. In fetuses with microphthalmia, the suprachiasmatic nuclei of the hypothalamus were generally reduced in size compared to the controls. In contrast, the suprachiasmatic nuclei were of normal size in $Ni(CO)_4$-exposed fetuses with normal eyes.

Ocular embryology and teratology in rodents are discussed in (*2, 3, 11*). The specificity of $Ni(CO)_4$ for induction of ocular anomalies appears to be unusual among known teratogenic agents. From a morphologic standpoint, the ocular anomalies that are induced in rats by $Ni(CO)_4$ closely resemble those that are induced in rats by maternal folic acid deficiency, and those that occur spontaneously in an inbred strain of mice (*3, 11, 12*). Hypoplasia of the suprachiasmatic nuclei of the hypothalamus, which was seen in rat fetuses with $Ni(CO)_4$-induced ocular anomalies, has also been observed in the inbred strain of mice with anophthalmia and microphthalmia (*13*). These findings support the hypothesis that normal formation of the suprachiasmatic nuclei may depend upon the presence of the eyes or the subadjacent optic axons during embryological development (*13*). From a toxicologic standpoint, there may be similarity between the mechanisms of teratogenesis by $Ni(CO)_4$ and by actinomycin D, since both of these compounds are potent inhibitors of DNA-dependent RNA-polymerase activity (*14*). Administration of actinomycin D to rats on days 5 to 10 of gestation has been reported to cause a variety of congenital malformations (*15*). However, ocular anomalies have only been prevalent when actinomycin D was given to rats on days 8 or 10 of gestation (*15*).

Ocular teratogenicity is probably a specific effect of $Ni(CO)_4$, rather than an effect of nickel compounds in general. Oral administration of divalent nickel salts to three generations of rats did not cause any congenital malformations (*16*). Furthermore, parenteral administration of $NiCl_2$ to pregnant Fischer rats on days 6 to 10 of gestation under the experimental conditions used in the present study did not cause any congenital anomalies (*17*). Studies of the metabolism of $^{63}Ni(CO)_4$ and $Ni(^{14}CO)_4$ in rats have shown that $Ni(CO)_4$ can cross the alveolar membranes without decomposition, and that the biological half-time of $Ni(CO)_4$ is approximately 0.5 hour (*18*). During 2 to 4 hours after exposure of rats or rabbits to $Ni(CO)_4$, the lung is a major excretory organ for $Ni(CO)_4$ (*18, 19*). Autoradiographic studies of adult rats after exposure to $^{63}Ni(CO)_4$ and $Ni(^{14}CO)_4$ have shown that $Ni(CO)_4$ can traverse the blood-brain barrier and enter the central nervous system (*20*). Therefore, we suggest that $Ni(CO)_4$ may cross the fetomaternal barriers in pregnant rats and enter the embryos without metabolic alteration. Further studies are needed to test this hypothesis and to define the pathological embryogenesis of the eye

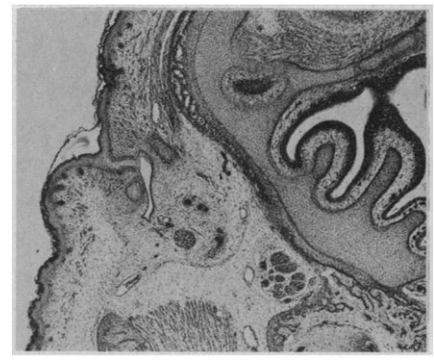

Fig. 3. Histologic section of the orbital area of an anophthalmic rat fetus that was exposed to $Ni(CO)_4$ on day 7 of gestation and killed on day 20 (hematoxylin and eosin, ×33).

which occurs after exposure of prenatal rats to $Ni(CO)_4$. Since 15 minutes of exposure to $Ni(CO)_4$ caused ocular malformations in rats, it seems prudent to control even brief occupational exposures of women of childbearing age to inhalation of $Ni(CO)_4$.

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Fertilization: A Uterine Glycosaminoglycan Stimulates the Conversion of Sperm Proacrosin to Acrosin

Abstract. *Acrosin is a proteinase required for mammalian fertilization, and in freshly ejaculated spermatozoa exists as an inactive zymogen, proacrosin. A factor present in uterine flushings of gilts stimulates the conversion of highly purified boar proacrosin to acrosin. Characterization of this factor indicates that its active component is a glycosaminoglycan.*

Acrosin (E.C. 3.4.21.10), a serine proteinase found in the acrosomes of mammalian spermatozoa, is used by spermatozoa for penetration of the zona pellucida of the ovum (*1*). The mechanism whereby proacrosin, the enzymatically inactive zymogen present at ejaculation (*2*), is converted to acrosin during the spermatozoa's residence in the female reproductive tract (*3*) is unclear. The work reported here shows that a glycosaminoglycan from porcine uterine flushings accelerates the conversion of highly purified proacrosin to acrosin.

Proacrosin was purified from ejaculated boar spermatozoa (*4*). Uterine fluid was obtained by flushing uteri with normal saline at laparotomy in gilts in which estrus was induced with pregnant mare serum gonadotropin and human chorionic gonadotropin (*5*). The generation of active acrosin from proacrosin was measured spectrophotometrically with *N*-α-benzoyl-L-arginine ethyl ester (BzArgOEt) at 253 nm (*6*).

Proacrosin is stable at *p*H 3.0, but when it is incubated at *p*H 7.0 it shows a sigmoidal conversion profile in which the time required for one-half maximum activity is 15 minutes ($t_{½}$). The $t_{½}$ is reduced to approximately 30 seconds when uterine flushings are included in the incubation medium (Fig. 1A). This acceleration of proacrosin conversion is dependent on the concentration of the uterine flushings (Fig. 1B). These results demonstrate the uterine flushings contain a factor that stimulates the conversion of proacrosin to acrosin.

Although proacrosin is converted to acrosin by trypsinlike proteinases (*4*), the uterine factor does not catalyze the hydrolysis of the synthetic trypsin substrate BzArgOEt, nor does the factor demonstrate proteolytic activity when measured with the general proteinase substrates Azocoll or Azoalbumin (lower limit of detection is 100 ng of trypsin equivalents per milliliter). Also, the activation of chymotrypsinogen and trypsinogen is not stimulated by the uterine factor. Furthermore, there is no demonstratable loss of the uterine factor's influence when it is incubated (24 hours at 37°C) with either trypsin (85 μg/ml) or pronase (83 μg/ml). Thus it is established that the uterine factor responsible for the stimulation of the conversion of proacrosin to acrosin is not a proteinase, and possibly not even a protein.

Anionic phospholipid vesicles also stimulate proacrosin conversion to acrosin (*7*). However, the uterine factor is not associated with subcellular organelles, nor is it a phospholipid, for it remains in the supernatant fluid after ultracentrifugation (2 hours at 150,000*g*), is not ether-extractable, and is resistant to digestion with phospholipase C (E.C. 3.1.4.3).

The ability to stimulate proacrosin conversion is rapidly lost when the uterine factor is incubated with testicular hyaluronidase (Fig. 2), an enzyme that specifically hydrolyzes the endo-*N*-acetylhexosamine bonds of hyaluronic acid

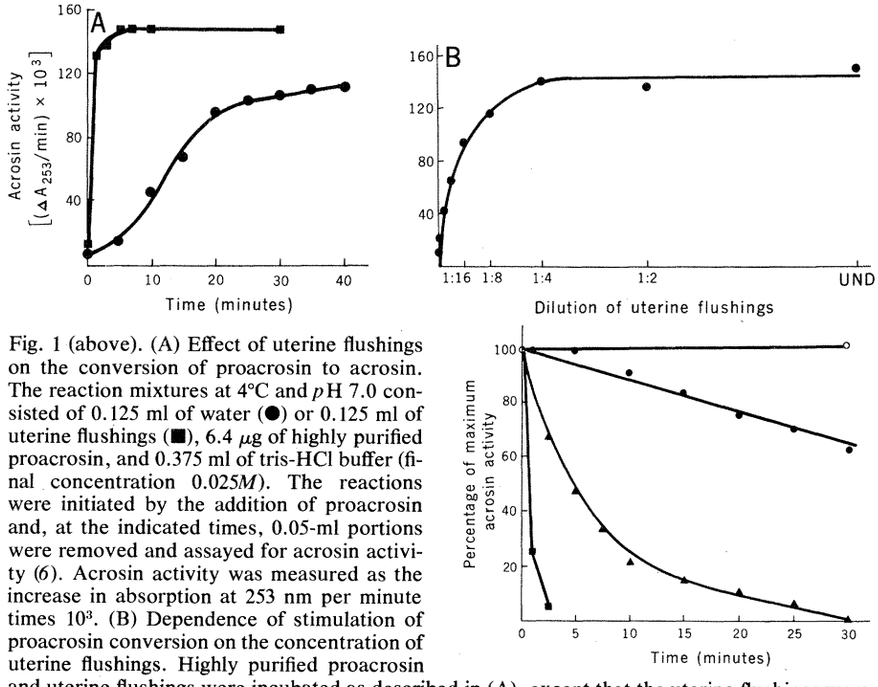

Fig. 1 (above). (A) Effect of uterine flushings on the conversion of proacrosin to acrosin. The reaction mixtures at 4°C and *p*H 7.0 consisted of 0.125 ml of water (●) or 0.125 ml of uterine flushings (■), 6.4 μg of highly purified proacrosin, and 0.375 ml of tris-HCl buffer (final concentration 0.025*M*). The reactions were initiated by the addition of proacrosin and, at the indicated times, 0.05-ml portions were removed and assayed for acrosin activity (*6*). Acrosin activity was measured as the increase in absorption at 253 nm per minute times 10^3. (B) Dependence of stimulation of proacrosin conversion on the concentration of uterine flushings. Highly purified proacrosin and uterine flushings were incubated as described in (A), except that the uterine flushings were diluted as indicated; *UND*, undiluted. After 3 minutes of incubation, 0.05-ml portions were removed and assayed for acrosin activity. Fig. 2 (right). Testicular hyaluronidase digestion of uterine factor. The reaction mixtures at *p*H 5.3 and 37°C consisted of 0.008*M* sodium acetate, 0.1 ml of undiluted uterine flushings, and testicular hyaluronidase, 5 μg (■), 0.5 μg (▲), 0.25 μg (●), and no hyaluronidase (○), in a total volume of 0.60 ml. Reactions were initiated by the addition of hyaluronidase and at the indicated times, 0.025-ml portions were removed and added to 0.11 ml of a *p*H 7.0 solution consisting of 0.08*M* tris-HCl and 2.89 μg of highly purified proacrosin, and were maintained at 4°C. After a 30-second incubation period, a 0.05-ml portion was removed and assayed for acrosin activity (*6*).

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