hours in flat-bottom plastic trays (Costar, Tissue Culture Clusters[24], Cambridge, Mass.) at a concentration of 1×10^6 cell/ml. The concentration of antigen was 10 μg/ml for BP, and 100 μg/ml for ovalbumin.

Portions of each cell preparation (0.2 ml) were also incubated in quadruplicate in 96-well flat-bottom microtiter tissue culture plates (1×10^6 cell/ml). After 2 days, 1 μCi of [^{3}H]thymidine (6.0 Ci/mmole, Schwarz-Mann) was added to each well, and 18 to 20 hours later the cells were harvested with a semi-automatic cell harvester (MASH II, Microbiological Associates). The radioactivity on the glass fiber discs was counted in a Packard Tri-Carb liquid scintillation counter. The stimulation index (S.I.) is equal to the ratio of the mean number of counts per minute of cultures containing antigen to that of cultures without antigen.

After a 3-day incubation period, with or without antigen, the cells intended for transfer were harvested and counted, and the number of phagocytic cells was determined. Cultured cells were then injected intraperitoneally into recipient strain 13 guinea pigs, which were weighed daily and checked for clinical signs of EAE. Positive animals developed clinical signs within 4 to 6 days. They were anesthetized when acutely ill. Negative animals were anesthetized 15 days after the injection of cells. The CNS tissues of all recipients were fixed for histologic examination (*6*).

The average phagocytic cell content of PEC was 63 percent. Plating for 2 hours reduced the content of phagocytes to 46 percent, with a recovery of 66 percent of the viable nonphagocytic cells. After 72 hours in culture, the average recovery of viable nonphagocytic cells was 44 percent of those put into culture. The final cell suspension injected into the recipient contained 69 percent viable nonphagocytic cells.

The cell transfer experiments are summarized in Table 1. Sensitized cells transferred after plating but without culture failed to transfer EAE even when 4×10^7 cells were used. In contrast, 1×10^7 of these cells incubated with BP for 72 hours transferred acute EAE to recipient animals. This result was completely reproducible, both clinically and histologically. Transfer of full-blown disease was also accomplished in two of three recipients with as few as 1×10^6 cells.

When cells from BP-sensitized animals were incubated without BP, and when cells from CFA-injected animals were incubated with BP, the disease was not transferred. The result in the latter case ruled out the possibility of active sensitization of recipients by BP nonspecifically bound to cells. The specificity of the effect of BP in culture on transfer of EAE was further confirmed by double sensitization of donors with BP and ovalbumin in CFA. Cells from these animals were subsequently cultured with BP or ovalbumin. Only the cells incubated with BP were capable of transferring disease.

The S.I. of cells from BP-sensitized animals, incubated with BP was variable, ranging from 1.7 to 9.6 with a S.I. for 15 experiments of 5.3 ± 0.6 (mean ± S.E.M.). Cells from doubly sensitized donors responded to both ovalbumin (S.I. = 13.9) and to BP (S.I. = 7.1).

From our S.I. data it is obvious that some BP-specific cells are proliferating in culture. This does not prove that the proliferating cells are the ones which transfer disease. In other immune systems it has been possible to separate cells that proliferate in vitro from the cells responsible for specific immune functions (*7*). It appears that the immunologic function of cells that proliferate in response to antigen is still poorly understood.

Recently, Panitch and McFarlin (*8*) described a system for adoptive transfer in the rat in which relatively small numbers of spleen cells could transfer EAE after culture with concanavalin A (Con A). Spleen cells not cultured with Con A were inactive. They postulated that the cells stimulated by the mitogen were "memory" T cells because they were still responsive to Con A after the donors had recovered from EAE. We have found that in vitro incubation with BP as well as in vitro incubation with Con A confers on sensitized rat spleen cells the ability to transfer EAE (*9*).

The method of transferring EAE with viable cells reported here provides an effective tool for examining immunologic and pathogenetic mechanisms of EAE in guinea pigs. Such studies were not feasible with the larger cell numbers previously required. Further studies are necessary to explain the effect of BP in culture and to identify the T cell subset (or subsets) responsible for the transfer of EAE.

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Rotaviral Immunity in Gnotobiotic Calves: Heterologous Resistance to Human Virus Induced by Bovine Virus

Abstract. *The possibility of immunizing human infants against rotaviruses, which cause severe dehydrating diarrheal disease, may depend on the use of a related rotavirus, derived from another animal species, as a source of antigen. To test the feasibility of this approach, calves were infected in utero with a bovine rotavirus and challenged with bovine or human type 2 rotavirus shortly after birth. Infection in utero with bovine rotavirus induced resistance to diarrheal disease caused by the human virus as well as the homologous bovine virus. These data suggest that the bovine virus is sufficiently related antigenically to the human type 2 virus to warrant further evaluation of the former as a source of vaccine.*

Rotaviruses are a major cause of serious, acute gastroenteritis in human infants up to 2 years of age (*1, 2*). Any attempt to immunize children should therefore be made early in infancy. Resistance to rotaviral disease appears to be mediated in man and in animals by local immunity at the epithelial surface of the small intestine (*3–5*). Because of the importance of local immunity, a live attenuated vaccine given orally is likely to be more effective than an inactivated

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vaccine administered parenterally. To date, the human rotaviruses have not been grown to high titer in tissue culture, and other related animal rotaviruses are the only strains available for evaluation as sources of vaccine. However, shared antigens have been demonstrated among rotaviruses derived from a variety of host species (*6*, *7*).

We have investigated the possibility that a calf rotavirus, Nebraska calf diarrhea virus (NCDV), can induce resistance to the human type 2 rotavirus. The NCDV had been passaged three times in gnotobiotic calves and was isolated in tissue culture by Mebus *et al.* (*8*). Because calves become refractory to disease produced by the human type 2 virus shortly after birth (*2*), it was not possible to evaluate the protective effect of NCDV infection at birth against challenge with the human virus 2 to 3 weeks later. We therefore infected the calves in utero with bovine virus and challenged them shortly after birth with the human type 2 virus, a method suggested by studies of Conner *et al.* (*9*) with *Escherichia coli* and of Newman (*10*) with coronavirus.

We first examined homologous immunity to NCDV. A 2 percent stool filtrate containing virulent calf rotavirus was inoculated into the amniotic sac of two bovine fetuses 3 to 5 weeks before they were born. Two additional fetuses were inoculated intraamniotically with veal infusion broth (VIB) containing 0.5 percent bovine serum albumin, the medium used to prepare the stool suspension. These four calves were delivered at term by cesarean section and maintained under gnotobiotic conditions (*11*). On day 1 after delivery each calf received an injection of virulent NCDV directly in the duodenum at laparotomy. The calves were observed daily for diarrhea, and serum was obtained from them at 3 weeks. The two calves exposed to NCDV in utero did not develop diarrhea when challenged at birth with the same virus preparation. Both animals showed rotavirus antibody in blood obtained from the umbilical cord at birth, and subsequently showed a considerable increase in this antibody at 3 weeks of age as measured by the enzyme-linked immunosorbent blocking assay (ELISA) with cross-reactive type 2 human rotavirus being used as antigen (*12*). The two control animals that had received VIB in utero developed diarrhea following challenge with NCDV at birth. Neither of these animals showed antibody in their blood at birth but within 3 weeks they both developed a serum antibody response. Although the number of animals studied was small, these findings suggest that protection against calf rotaviral disease is induced by exposure to homologous virus in utero.

Table 1. Immunity to NCDV and HRV-D in gnotobiotic calves exposed to NCDV or veal infusion broth (VIB) in utero.

Number of calves tested	Inoculum administered		Number of calves			
	In utero	Postnatal	With diarrhea after postnatal challenge	Shedding virus	With antibody at birth*	With fourfold or greater increase in antibody response at 3 weeks*
2	VIB	NCDV	2	NE†	0	2
2	NCDV	NCDV	0	NE†	2	2
8	None or VIB‡	HRV-D	7	8	0‖	7‖
5	NCDV	HRV-D	0	4	5	4

*Antibody measured by ELISA blocking with type 2 human rotavirus. †Not examined. ‡Four animals received VIB in utero. ‖Serum not available from one animal at birth.

We next determined whether calves exposed to NCDV in utero would also show resistance to disease caused by human type 2 rotavirus (strain HRV-D). Virulent NCDV, or the VIB control fluid used in the initial experiment, was inoculated directly into the amniotic sac of nine fetal calves 2 to 24 weeks before delivery. These calves, as well as four others which were not inoculated in utero, were delivered at term by cesarean section and maintained under gnotobiotic conditions. These calves were inoculated intraduodenally on days 1 or 2 after delivery with a suspension of human rotavirus previously shown to produce disease in newborn gnotobiotic calves (*13*). The strain HRV-D used was in a 2 percent fecal filtrate derived from an experimentally ill calf (No. 75-42) which had received HRV-D passaged once previously in gnotobiotic calves. Two different virus suspensions derived from this calf were used for challenge and both suspensions had produced diarrhea in newborn calves. Serum was collected at birth from all but one calf and again 2 to 3 weeks later from all animals. Fecal samples were collected for up to 9 days after administration of HRV-D.

None of the five calves that had received NCDV in utero developed diarrhea when challenged with HRV-D shortly after birth. In contrast, seven of eight animals that had not been exposed in utero to NCDV developed diarrhea when challenged with the HRV-D inoculum. The ELISA procedure was used to evaluate the immunologic response of these animals (*12*). Each of the five calves that had prior exposure in utero to NCDV showed rotavirus antibody in serum obtained from the umbilical cord. Four of these five calves developed a fourfold or greater increase in antibody after postnatal challenge with HRV-D. None of seven control animals had rotavirus antibody in their serum, but each developed a significant antibody response 3 weeks later. The fecal samples collected after challenge with HRV-D were also examined for rotavirus antigens by means of ELISA (*14*). Four of the five animals exposed to NCDV in utero shed rotavirus for 1 to 4 days after the postnatal challenge with HRV-D. In contrast, each of the eight control animals shed virus for 3 to 9 or more days after challenge with HRV-D.

Human rotavirus (type 2) was shown previously to infect and induce diarrhea in newborn monkeys, calves, and piglets (*2*). While our study was in progress, the existence of two distinct serotypes of human rotavirus was established (*15*), and longitudinal surveillance of infants and young children revealed that infection with type 1 virus did not protect against disease caused by the type 2 virus (*16*). Also, concurrently, cross-neutralization tests in tissue culture, with immunofluorescence being used to detect foci of infection, indicated that antibodies against bovine rotavirus were 16 times less effective in neutralizing human rotavirus than homologous virus (*17*).

On the basis of these newer observations one would not anticipate that the bovine rotavirus would induce resistance to the human virus, especially since one human rotavirus serotype does not protect children against the other. Nonetheless, the present study indicates that bovine rotavirus protects calves against human (type 2) rotavirus. One factor responsible for this success may have been the relatively short interval between infection with bovine rotavirus and challenge with the human virus (2 to 14 weeks), whereas sequential infection of children with different serotypes usually occurs after a longer interval (*16*).

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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