Interferon Administered Orally: Protection of Neonatal Mice from Lethal Virus Challenge

Abstract. *Interferon was identified in the milk of mice injected with an interferon inducer. The kinetics of interferon appearance in serum and in milk were similar, but maximum concentrations in milk were 10 to 20 percent of those in serum. Interferon administered orally to neonatal mice was detected in their serums. Significantly more newborns survived an oral challenge with vesicular stomatitis virus when interferon had been induced in the lactating mothers.*

Although it is well known that interferon (IF) can contribute to the protection of virus-infected hosts, it has not been shown to be effective when administered orally. We devised two systems to implicate orally administered IF as a part of the defense mechanism of virus infections of neonatal mice: (i) mice were given mouse IF orally from serum or tissue culture; (ii) newborns suckled mothers previously injected with IF inducers. In such induced mothers, a viral inhibitor with the biophysical and biological properties of IF (*1*) was found equally distributed in cream and skim milk. We determined the kinetics of the appearance of IF in skim milk and in serum after injecting the mice with Newcastle disease virus exposed to ultraviolet radiation (UV-NDV). Interferon reached maximum concentrations in the milk (500 unit/ml) and in the serum (6000 unit/ml) at about the same time (8 hours). In four experiments, the maximum IF concentration in the milk was 10 to 20 percent of the maximum concentration in the serum; in two of these experiments, IF was observed in the milk 15 hours after it had disappeared from the serum.

To determine whether the orally administered IF reached the circulation, we fed IF to groups of 35 to 40, 5-day-old mice every hour for 7 hours. They were exsanguinated 1 hour after the last feeding, and the blood was pooled and assayed for interferon with the plaque reduction test (*2*) on mouse fibroblasts (L-929); vesicular stomatitis virus (VSV) was used as the challenge virus. The recovery of orally administered IF in infant mice is shown in Table 1. Exogenous IF derived from mouse cell cultures or from mouse serum was recovered from the serums of 5-day-old mice. These findings indicated that orally administered IF could reach the circulation of suckling mice from the gut. However, the IF fed to the mice was not pure, and the possibility existed that impurities, or possibly the IF itself, could have induced the circulating IF. To ascertain that the circulating IF originated from the gut and not from an induced synthesis, mice were fed rabbit IF (*3*). Mouse IF was inactive on rabbit cells; thus, the detection of rabbit IF activity in the serums of neonatal mice would demonstrate that it originated from the passively administered source. If the IF were induced as a response to the orally administered IF, it would be inactive in a rabbit cell assay system. Suckling mice (5 days old) were fed rabbit IF on the same regimen as described above and were bled 1 hour after the last feeding. The serums from the neonatal mice were assayed for IF concentration as described above, except that the IF assay system consisted of cells from rabbits (primary kidney), rather than from mice. Interferon with the biological characteristics of rabbit IF was detected in the serums of these mice (Table 1).

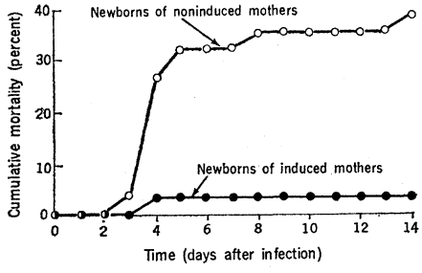

Fig. 1. Influence of interferon in lactating females on the mortality of newborns infected with VSV. Eighty 5-day-old neonatal mice (ten per mother) were inoculated orally with approximately one LD_{50} of virus (in 0.05 ml). Half of the mothers were injected intravenously with UV-NDV 6 hours before the neonatal mice were infected. Deaths were recorded twice daily.

Similar results have been obtained with 8-day-old mice, but IF was not detected in the serums of adult mice when it was administered orally. This failure to detect IF in adult serum may be due to (i) digestive enzymes present in the mature mouse, but absent or in lower concentration in the neonatal intestinal tracts; or (ii) an IF dose that was too small. The fact that IF can be detected in the blood of neonatal mice in low concentrations suggests that exogenous IF may augment the natural defense mechanisms of newborns. The half-life of circulating IF has been calculated to be about 11 minutes (*4*); it is not surprising, therefore, that the concentration of IF in serum was low.

Because the orally administered IF reached the circulation, we designed experiments to determine whether orally administered IF would protect newborns from virus infection. Mice born within 24 hours of each other were selected, and litter size was reduced to ten nurslings per mother. Several different methods of administration of IF were tested, for example, by micropipette, gavage, or induction in lactating females with UV-NDV (injected intravenously) that induced approximately 10,000 to 20,000 international units (I.U.) of IF per milliliter of serum in these females. The neonatal mice were challenged with one to four lethal doses, 50 percent effective (LD_{50}), of VSV, injected subcutaneously. Experiments with 80 newborns each demonstrated that there was little or no protection afforded as compared to controls that did not receive IF. However, animals were significantly protected when the IF and the virus were administered by the same route, that is, orally. For example, 6 hours before

Table 1. Detection of IF in serum after oral administration. Mouse L cell (L-929) IF was produced with live UV-NDV as inducer by a modification of the method of Youngner *et al.* (*7*). Mouse serum IF was prepared as described (*8*). Both IF preparations were acidified and kept at *p*H 2 for 5 days at 4°C; they were then adjusted to approximately *p*H 7.0 and filtered (0.45 μm) before being assayed and fed. Rabbit IF was prepared as described by Vilček and Ng (*9*). Recovery of IF was calculated from an assay of 1 ml of serum recovered from 40 neonatal mice. Rabbit IF recovered from neonatal mice was assayed on primary rabbit kidney cells.

Exogenous IF source	IF administration (total units per mouse)*	IF recovery (total units per mouse)†
Mouse cell culture	3850	4.0
Mouse serum	1400	2.0
Rabbit cells (primary kidney)	4200	32.0

* Assuming all IF administered was swallowed. † Assuming that IF is distributed uniformly in the extracellular compartment that is approximately 0.2 ml of a 1-g mouse.

newborns were infected with VSV, mothers were inoculated with UV-NDV (*5*) which induced peak titers of approximately 50,000 to 60,000 I.U. of IF per milliliter of serum. The neonatal mice were then challenged orally with one LD_{50} of VSV, and a 35 percent reduction in deaths was observed ($P < .001$) (Fig. 1). Similar results were noted in two other tests.

Korsantiya and Smorodintsev (*6*) have reported that the offspring of mice that had been stimulated by IF inducers just before giving birth were more resistant to viral challenge than newborns from (unstimulated) control mothers. They attributed the resistance solely to transplacental transfer of IF. Their data show that maternal serum IF was maximal at the time the suckling infants were challenged. It is probable that IF was in the milk and that it contributed to the protection of the newborns.

We have demonstrated that IF administered orally can favorably influence the course of certain viral infections in newborns. Possibly, if higher doses of IF had been given, the protection could have been increased. Our results may also indicate that orally administered IF may be of value to human newborns.

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4 February 1972; revised 10 April 1972

Axonal Transport of Tritium-Labeled Putrescine in the Embryonic Visual System of Zebrafish

Abstract. *The transport of [^3H]putrescine is demonstrated by autoradiography in the retino-tectal tract of* Brachydanio rerio *embryos. Transport of [^3H]putrescine appears to be more rapid than that of tritium-labeled protein and is not inhibited by a colchicine effect on axonal neurotubules as is protein transport. The radioactivity transported to the brain is found, on electrophoresis, in the putrescine fraction.*

Silver grains in autoradiographs with tritium-labeled putrescine (1,4-diaminobutane) and its metabolites is present in a higher concentration in nerve tracts than in the adjacent gray matter (*1*). We thus considered the possibility of axonal transport to account for this high concentration in the nerve tracts. Axonal transport of protein (cellulifugally) containing incorporated radioactive labeled amino acids (*2*) has been documented, and there have also been reports of transport of glycoproteins (*3*), carbohydrates (*4*), phospholipids (*5*), RNA (*6*), and noradrenaline (*7*). Naturally occurring amino acids and synthetic amino acids such as cycloleucine are not transported (*8*). There are no reports in the literature of transport of amines, among which are very common naturally occurring substances including putrescine, and spermidine and spermine, the so-called polyamines (*9*). With the use of a system described previously (*10*), we demonstrated axonal transport of putrescine and compared its characteristics to axonal transport of protein.

Table 1. Increase in grain counts in contralateral tectum (CT) over background in ipsilateral tectum (IT) of *Brachydanio rerio* embryo following intraocular injection of [^{3}H]-putrescine preceded by colchicine 1 hour earlier. The grain counts are means of a minimum of five sections.

CT	IT	Increase (%)
	[^3H] *Putrescine, 3 hours*	
1620	1169	38.6
	[^3H] *Putrescine, 24 hours*	
2187	1587	37.8
	[^3H] *Putrescine + colchicine, 3 hours*	
2046	1482	38.1

We synthesized [2,3-^{3}H]putrescine dihydrochloride, specific activity 744 mc/mmole, by catalytic tritiation of 1,4-diaminobutine (*11*). About 0.005 μl (5 nc) of a solution of 5 mg of the labeled putrescine in 1 ml of Sorensen phosphate buffer, *p*H 7.4, was injected into one eye of 7-day-old *Brachydanio rerio* (zebrafish) embryos through a glass micropipette with a micromanipulator. Embryos used for observations of putrescine axonal transport were killed by immersion in 3.5 percent aqueous glutardialdehyde at 0.5, 1, 1.5, 2, and 24 hours after injection. After 2 hours in glutardialdehyde they were further fixed with osmium tetroxide and embedded in Epon 812 containing 1 percent 2,5-diphenyloxazol scintillator (*12*) to shorten exposure time. Transverse semithin and ultrathin sections of the brain were made so that the entire retino-tectal tract, from the retina across the chiasma to the optic tectum, was generally present in the section. Autoradiographs were made from the semithin sections by the dipping method with undiluted Ilford L4 emulsion. After an exposure of 1 to 3 days, they were developed (Kodak D19b) for 6 minutes, fixed, and examined unstained under phase contrast and darkfield microscopy.

The autoradiographs revealed a heavy labeling of the injected eye and the contralateral optic tract to the tectum as early as 30 minutes after putrescine injection (Fig. 1). Because of the relatively high background radiation carried by blood to the ipsilateral tectum, the visual indication of transport to the contralateral tectum was tested and verified by grain counts to ascertain the percentage of label over the background in the side labeled by axonal transport. There was an increase of about 38 percent in grain counts in the tectum contralateral to the eye injected with putrescine (Table 1).

Thirty minutes after injection, the transported label had already reached the contralateral tectum. The distance from the ganglion cell layer of the retina to the contralateral tectum is an average of 0.318 mm; therefore, the transport rate is at least 15.3 mm/24 hours. The rate is actually likely to be faster, since at 30 minutes the contralateral tectum was already labeled. In a study with the same transport model (*10*), we noted that transported labeled protein reached the contralateral tectum in slightly less than 1 hour. The more rapid transport of putrescine