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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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

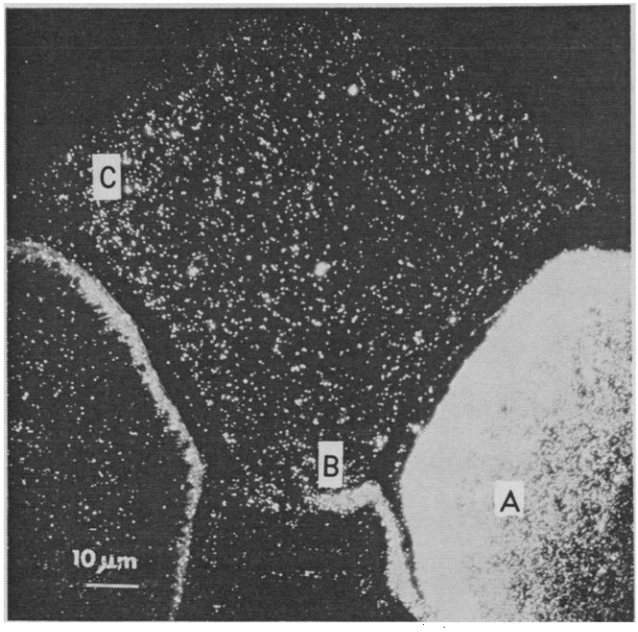

Fig. 1. Autoradiograph of a cross section through a 1-week-old *Brachydanio rerio* brain showing the labeled retino-tectal tract. Right eye (*A*) injected with [^{3}H]putrescine 30 minutes before the animal was killed. Right optic nerve (*B*) and contralateral optic tectal region (*C*) show transported label.

might indicate that another mechanism was involved. In our previous study, we found that transport of labeled protein was blocked by prior injection of the eye with colchicine, a result indicating that axonal transport of protein may be related to the integrity of the neurotubular system of the axon (*13–15*). We therefore injected a group of embryos, intraocularly, first with colchicine (*10*) and then with putrescine. The same dose of colchicine as used previously was injected as this amount had proved effective in inhibiting protein transport in our model.

Colchicine (about 10^{-4} μl of a 0.2 percent solution in 20 percent ethanol, adjusted to *p*H 7.4 with 0.1*M* phosphate buffer) was injected into only one eye of 7-day-old embryos. This was followed 1 to 2 hours later by the injection of [^{3}H]putrescine into the same eye. Three hours after this, the embryos were killed by fixation and were prepared for autoradiographic analysis. The autoradiographs revealed that transport of the label to the contralateral tectum in the experimental animals was in no way different from that in the group not treated with colchicine. Grain counts were almost identical in the two groups (Table 1). To verify that an effective dose of colchicine was present in this experimental group, we looked for evidence of metaphase mitosis arrest (*13*). In the embryos injected with putrescine but not colchicine, 20 consecutive midbrain sections revealed a total of eight mitotic figures by phase contrast examination of the semithin sections. In a colchicine treated embryo, 20 consecutive sections revealed 125 cells in the metaphase stage of mitosis, a demonstration that sufficient colchicine was present to disturb the integrity of spindle protein, which is highly analogous to neurotubular protein (*14*).

Axons in the retino-tectal tracts of the putrescine and of the colchicine and putrescine treated embryos were examined by electron microscopy. No significant abnormalities were noted in the neurotubules of the putrescine-treated group.

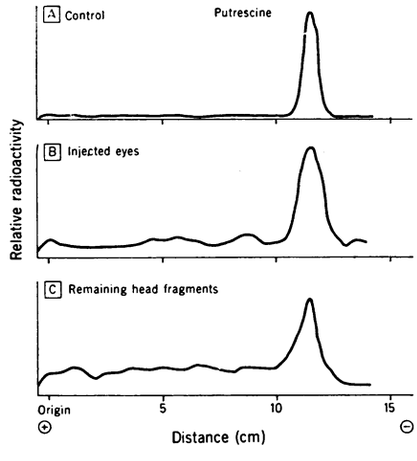

Fig. 2. Distribution of radioactivity after electrophoresis of aliquots of HCl extracts of ten pooled extirpated, injected eyes (B) and remaining ten pooled head fragments (C); 5 nc of [^{3}H]putrescine was used as a reference standard (A). The relative high background over the baseline in (B) and (C) is the result of contamination by labile tritium from tritium water, a result of oxidative metabolism of [^{3}H]putrescine.

However, in the group treated first with colchicine, many of the axons were distended, and the number of neurotubules present in these distended axons was decreased, as described (*15*). However, there was no increase in neurofilaments in these altered axons, but instead a large amount of fine granular material was present; perhaps this is related to the relatively early embryonic stage of the animals, at which time axons normally contain very few neurofilaments (*16*).

To show that the transported labeled substance is putrescine and not a metabolite, we injected one eye of each of ten 7-day-old embryos with putrescine. After 3 hours, the ten injected eyes were extirpated with a micromanipulator. The heads with the remaining noninjected eyes were separated from the rest of the embryos by cutting them just distal to the hindbrains. It was necessary to analyze the eyes and the heads separately to see (i) if putrescine remained unaltered after 3 hours in the eyes, and (ii) that the radioactive material in the head and brain was not hidden by the large amount of labeled putrescine remaining in the injected eye.

The ten eyes were pooled as were the ten heads and they were treated separately. Each pooled group was homogenized in a glass homogenizer (Potter-Elvehjem) containing 0.75 ml of ice-cold 0.6*N* HCl. The homogenate was centrifuged at 800*g* for 10 minutes, and the resultant precipitate was then homogenized in 0.25 ml of 0.6*N* HCl. Ethanol was added to the combined supernatants to a final concentration of 70 percent. This mixture was then stored at −18°C for 12 hours to complete the protein precipitation. After centrifugation, the total hydrochloric acid extract was applied to 20 by 20 cm (Eastman Kodak K 301 V) silica gel thin-layer plates for electrophoresis. The application of material to the plates was made in 3-cm streaks at a distance of 2 cm from the plate edge with the aid of the autoliner (Desaga). The plates were sprayed with pyridine, acetic acid, citric acid buffer (*17*) at *p*H 4.8 and subjected to electrophoresis at 20 volt/cm for 90 minutes in a Desaga TLE chamber. Air-dried electrophoretograms were scanned with a radioactivity scanner and were then cut into 5 by 40 mm strips along the length of the path. The layers removed from the strips were subjected to radioactivity measurements by liquid scintillation counting. This electrophoretic proce-

dure was developed for the identification and separation of amines and polyamines (*17*) and has proved to be reliable (*1*, *18*).

Results of the analysis of the electrophoretograms of the eye and head extracts are shown in Fig. 2; the largest amount of transported radioactivity is contained in the putrescine fraction. The protein precipitate fraction was analyzed for radioactive content by liquid scintillation counting and was found to contain insignificant amounts of activity.

Since putrescine and its related polyamine metabolites (*19*), spermidine and spermine, are found in relatively high amounts in the nervous system (*20*) of all animals studied so far, the demonstration of axonal transport of putrescine is of special significance. We have demonstrated that axonal transport of putrescine is probably not dependent on the integrity of the neurotubules. Although we have shown the axonal transport of exogenously administered putrescine, this does not mean necessarily that putrescine axonal transport is a normal physiological phenomenon.

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Low Internal Conductivity of Aplysia Neuron Somata

Abstract. *The internal conductivity of* Aplysia *neuron somata was measured by passing constant current pulses across a calibrated four-electrode array. The intracellular medium is less than one-tenth as conductive as seawater. The low conductivity probably results from structured cell water since ions are present in quantity and do not appear to be bound.*

Most living cells are so small that a direct measurement of the electrical conductivity of the internal medium is not possible. The most notable exception is the giant axon of the squid, for which values of axoplasmic conductance (the inverse of resistance) between 0.7 and 1 times that of seawater have been obtained (*1*). In addition, the internal conductance of frog muscle fibers has been reported to be one-half to one-third that of saline (*2*). Schwan and Li have measured the conductivities of packed tissues at ultrahigh frequencies and report that mammalian skeletal and heart muscle, liver, kidney, and lung tissues all have internal conductivities considerably less than that of the external medium (*3*). For red blood cells Pauly and Schwan have shown (*4*) that the internal conductivity was less than half that expected from the known concentration and mobility of the internal ions, even when allowance was made for the volume concentration of hemoglobin.

We have recently reported a new technique for the measurement of intracellular conductivity in which a single metal microelectrode at a current frequency of 100 khz is used (*5*). With this method it is possible to measure the conductivity of a thin layer of fluid at the tip of the electrode. We found that, although the conductivity of squid axoplasm is near to that of seawater, the internal conductivity of *Aplysia* neuronal somata is only about 5 percent of that of the external medium. In the study reported here we have measured the internal conductivity of *Aplysia* neurons by an entirely independent method as a test of both the technique used and the conclusions reached in our earlier study.

This method was developed by Li *et al.* (*6*) for measurement of the resistivity of brain tissue. Four electrodes were fixed with dental cement in a linear array. The electrodes were approximately equidistant, and the separation between the outermost electrodes was about 500 μm. Constant current pulses were passed between the outer two electrodes, and the voltage drop was recorded between the center two electrodes. Since the distance between electrodes is fixed and the current was constant, the voltage recorded is a function only of the resistance of the medium between the recording electrodes.

In order to use this technique within single cells we made arrays of glass-insulated platinum metal microelectrodes (*7*), each coated with a layer of platinum black. The individual electrodes had a diameter of about 1 μm at the tip and were about 12 μm in diameter at the point where the insulation began, approximately 15 μm from the tip. The array was calibrated in dilutions of artificial seawater of known conductivity, with all recordings made against a Ag-AgCl reference wire; there is a linear relationship between conductivity and concentration over this range (*5*). In different experiments the current pulses used varied between 1 and 10 μa and their durations varied between 0.5 and 1.0 msec. As long as there was a good coating of platinum black on the electrodes, they remained stable at low frequencies (usually six pulses per minute). If the voltage changed with time, the electrode arrays were discarded or replatinized.

These arrays were used to measure the internal conductivity in three different identified neurons of *Aplysia*: the giant cell located in the left pleural ganglion and cells R_2 and L_6 [terminology from (*8*)] in the visceral ganglion. These cells may be in excess of 800 μm in diameter in large animals. Ganglia were removed from the animal, pinned to paraffin in a Lucite chamber, and maintained under flowing seawater and controlled temperature. The connective