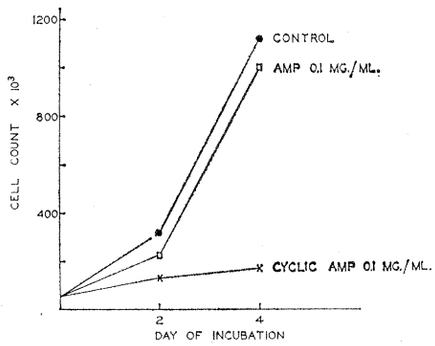

Fig. 1. Effect of 3′,5′-AMP and 5′-AMP on growth of strain L cells.

AMP count was 90 percent of the control at day 2 and 101 percent of the control at day 4. These data suggest that the inhibition of the cells is reversible and that no permanent damage occurs as a result of the treatment with 3′,5′-AMP.

N^6-2′-*O*-Dibutyryl-adenosine 3′,5′-monophosphate (dibutyryl-3′,5′-AMP) has a greater activity than 3′,5′-AMP (*10–12*). This increased potency is due partly to a greater resistance to hydrolysis by phosphodiesterase and perhaps to increased penetration into the cell. For this reason, the growth inhibiting activity of 3′,5′-AMP was compared with that of dibutyryl-3′,5′-AMP (Table 2). The failure of the dibutyryl derivative to inhibit the growth of L cells was unexpected; therefore, another lot of this compound was obtained and tested in the same manner with similar results.

Table 1. Dose response of strain L cells to 3′,5′-AMP. Eight determinations were made at each concentration of 3′,5′-AMP. Results are expressed as percent of the control cell count.

Conc. (mg/ml)	Response (%)	
	Day 2	Day 4
0.10	58	16
.07	60	23
.04	61	44
.01	105	103

Table 2. Comparison of inhibition of strain L cells by 3′,5′-AMP and by dibutyryl-3′,5′-AMP. Sample 1, Boehringer-Mannheim (lot 6068310). Sample 2, Boehringer-Mannheim (lot 06477308). Each result is the average of four separate determinations. Results expressed as percent of the control cell count.

Conc.	Inhibition (%)	
	Day 2	Day 4
	3′,5′-AMP	
0.12	66	54
.30	48	19
	Sample 1. Dibutyryl-3′,5′-AMP	
0.12	100	84
.30	84	67
	Sample 2. Dibutyryl-3′,5′-AMP	
0.12	85	79
.30	76	58

Adenosine 3′,5′-monophosphate appears to mimic at least one type of activity on cell cultures observed with steroids, namely, growth inhibition. The inhibition, like that of the steroids, is reversible, and upon removal of the 3′,5′-AMP the cells grow at a normal rate. The effect of 3′,5′-AMP on cells is unusual because it is generally believed that phosphorylated intermediates are not transported without first being dephosphorylated (*13*). It would appear that 3′,5′-AMP is not enzymatically altered before entering the cell since 5′-AMP, the product of phosphodiesterase activity on 3′,5′-AMP, does not have any activity on cells in culture.

Dibutyryl-3′,5′-AMP has a greater activity than 3′,5′-AMP on blood glucose (*10*), stimulation of lipolysis (*11*), amylase secretion in rat parotid cells (*14*), and relaxation of guinea pig tracheal chain preparation (*12*). This greater activity of the dibutyryl derivative is generally ascribed to its resistance to hydrolysis by phosphodiesterase and to increased penetration of the cell. However, two separate preparations of the dibutyryl-3′,5′-AMP failed to inhibit the growth of L cells. It seems possible that the dibutyryl-3′,5′-AMP is not active until converted to 3′,5′-AMP and that the enzyme required for this conversion is not present in L cells.

The minimum concentration of 3′,5′-AMP required to inhibit the L and HeLa cell cultures is approximately 0.12 mmole/liter. The concentration of 3′,5′-AMP required to increase the release of free fatty acids is 1.3 mmole/liter (*11*); to produce estrogen-like effects in isolated uteri 5 to 10 mmole/liter (*5*). Although, 3′,5′-AMP is ineffective on the guinea pig tracheal chain, dibutyryl-3′,5′-AMP (1.02 mmole/liter) is required to relax the guinea pig tracheal chain. The lower concentration of 3′,5′-AMP required for inhibition of cell multiplication suggests that it might act as a regulator of cell growth as well as an intermediary in hormone action.

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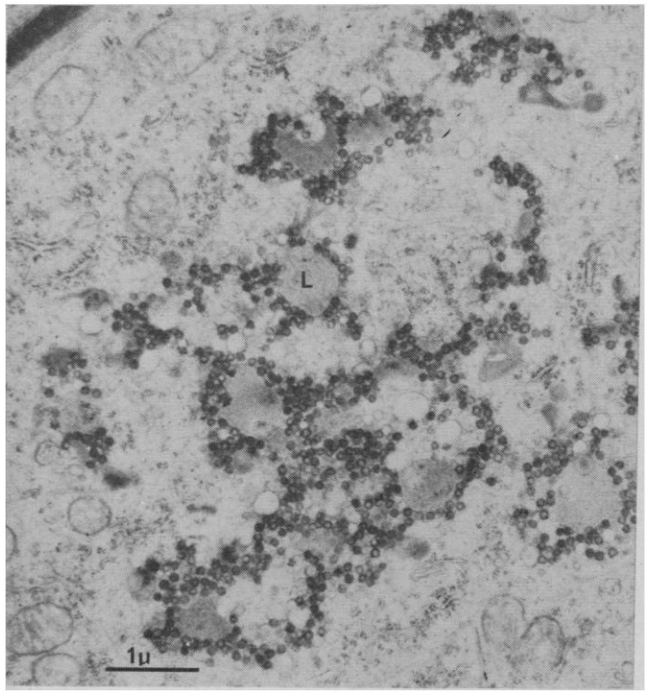
Virus-Like Particles in the Spiral Ganglion of the Guinea Pig Cochlea

Abstract. *Double-walled spherical particles 100 millimicrons in diameter were accidentally discovered in the cytoplasm of spiral ganglion neurons of apparently healthy guinea pigs with normal startle responses. These particles in some ways resemble viruses of the herpes group and may represent a latent form of neuronal infection.*

During an electron microscopic study of the normal spiral ganglion in guinea pigs, we discovered unusual spherical particles in some ganglion cells. The neurons were otherwise identical to uninvolved cells. Each was wrapped with several layers of myelin, and contained the usual cytoplasmic and nuclear elements of spiral ganglion neurons described in the literature (*1, 2*). The spheres were grouped around relatively large homogeneous lysosome-like bodies as seen in Fig. 1. Their cores measured from 50 to 60 mμ, and often appeared empty. Outside a dense layer bounding the core was a clear space of 10 to 15 mμ and an outer dense layer; the outer diameter averaged 100 mμ. Some particles, as illustrated in Fig. 2, were ovoid. None were found inside cell nuclei. Their appearance best fits the textbook description of the nuclear form of one of the herpes virus



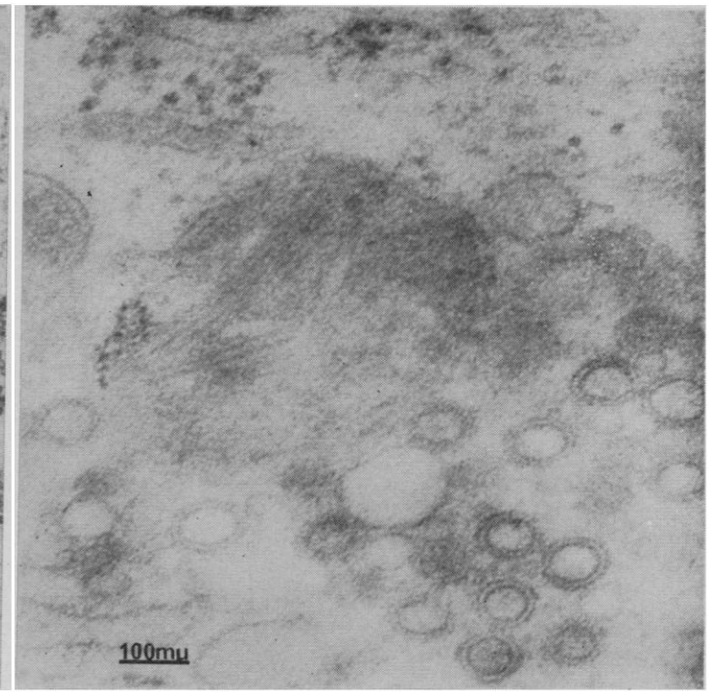


Fig. 1 (left). Myelinated neuron of guinea pig spiral ganglion. Numerous 100-mμ spheres were found in the cytoplasm, many of them clustered around lysosome-like (*L*) particles. The neurons otherwise appear normal. Fig. 2 (right). A single outer layer surrounded the particles that were found only in neural cytoplasm. Although a few of the virus-like particles had solid cores, most appeared to be only partly filled or empty.

group (*3*). The extra layer that ordinarily surrounds herpes virus in cytoplasm was not present.

After accidentally discovering several spiral ganglion cells with these herpes-like "viruses" from a single specimen, we studied a number of other specimens from healthy guinea pigs, with normal startle responses to auditory stimuli, chosen at random from our animal colony. We examined several hundred sectioned ganglion cells from eight animals and encountered another spiral ganglion with several infected neurons. The particles were identical to those found in the first animal. The spiral ganglion of only one side was infected in each animal. The involved cochlea of each was otherwise normal, a survey with a light microscope and spot-checking by electron microscopy showing no evidence of hair cell degeneration. Conventional processing for electron microscopy was used. Glutaraldehyde was perfused vascularly, and the dissected specimens were then post-fixed in osmium tetroxide. Epon was used for imbedding and Reynold's lead citrate (*4*) and uranyl acid for staining.

Herpes simplex, vericella-zoster, and cytomegalovirus are well-known members of the herpes group of DNA viruses. Otitic herpes had been previously recognized for many years when Ramsey Hunt described the syndrome, named after him, of geniculated ganglion herpes zoster in 1907 (*5*). Facial paralysis and cutaneous auricular vesicles are the most common manifestations, although the area of skin involvement may vary. Mild herpetic infection of the auditory ganglion produces tinnitus, but varying degrees of loss of hearing, from mild temporary to severe permanent, have been described in patients with the Ramsey Hunt syndrome. The clinical features of this ailment have been well documented, but the virus has never been demonstrated in the auditory ganglion. Mumps and measles viruses can produce impairment of hearing in adult humans (*6*), and rubella is a common cause of congenital deafness. Deafness can also accompany or follow an acute upper respiratory tract infection, creating the possibility that one or more "common cold" virus can infect the inner ear or 8th nerve (*7*). As Schuknecht *et al.* (*8*) demonstrated pathologically, some kinds of viruses cause degeneration of the organ of Corti while sparing the nerve and ganglion. Because of its neurotropism, the most likely target for aural herpes infection would seem to be neurons of the spiral ganglion, rather than hair cells or other organ of Corti components.

Until the infective nature of these particles can be demonstrated by tissue culture or other techniques, their nature and classification will not be established. Their core of 50 to 60 mμ and outer layer of 100 mμ in diameter morphologically resemble those of the herpes group viruses as they usually appear in cell nuclei. Cytomegalovirus often has an incompletely filled core (*9*), as these particles do. Previous descriptions of herpes group virus have been based on observations of multiplying, active viruses, although latency is a well-known feature of these viruses (*10*). The missing outer layer of cytoplasm particles, and "empty" cores exhibited by these particles, may be dormancy characteristics. The healthy appearance of the neurons could be explained as a latent infection. Association with lysosome is another feature shared with herpes group viruses (*11*).

Particles of this type were found in guinea pigs exposed to acoustic trauma and interpreted as a form of degenerative response (*2*). Little is known about the viral impairment of hearing in animals, and none of the many papers about virus-caused disease of the inner ear in humans have reported visualization of virus particles.

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Photoregulation of an Enzymic Process by Means of a Light-Sensitive Ligand

Abstract. *A specific inactivator of chymotrypsin,* p-*azophenyldiphenylcarbamyl chloride, exists as two geometric isomers,* cis *and* trans, *which are interconvertible by means of light. The* cis-*isomer is five times more reactive than the more stable* trans-*isomer, and is obtained by exposure of the latter to light of 320 nanometer wavelength. The* trans-*isomer can be regained by exposure of the* cis-*isomer to light of 420 nanometer wavelength. This interconversion can be made to occur in aqueous solution in the presence of the enzyme under conditions in which the* trans-*isomer reacts relatively slowly with chymotrypsin. Thus, it is possible to regulate the rate of inactivation of chymotrypsin by using light of the appropriate wavelength. This system is presented as a model for some of the light-sensitive metabolic systems present in living organisms.*

Photosensitive and photoregulated processes, such as photoperiodicity, photoreactivation, and phototaxis, function in many living systems (*1*). Although it is assumed that the effect of light is ultimately on some enzymic processes, the mechanism by which the radiant energy is utilized in the chemical response is largely obscure. There is agreement that pigments, in particular certain carotenoids, may be involved as mediating agents, but the manner in which they might influence or regulate an enzymic process is not known. We now describe a system which might serve as a model. In this system, an enzymic process, in itself insensitive to light, can be made subject to photoregulation by means of a small, light-sensitive effector molecule.

Diphenylcarbamyl chloride (DPCC) is a specific inactivator of chymotrypsin (*2*); inactivation occurs because of a reaction between DPCC and an essential serine residue at the active site of the enzyme. In an attempt to prepare a chromophoric analogue of DPCC, we synthesized the reagent *p*-azophenyldiphenylcarbamyl chloride (PADPCC) (*3*), which as a potent inactivator of chymotrypsin, is even more active than DPCC. Recently, it was noted that the absorption spectrum of PADPCC and its reaction with chymotrypsin were influenced by light. It soon became apparent that PADPCC was photochromic (*4*), undergoing a reversible change in structure under the influence of light. In the case of azo derivatives such as PADPCC, the change caused by light involves interconversion of *cis-trans* geometric isomers, that is, rearrangements around the N=N bond. Although unequivocal identification of the isomer is made by dipole moment measurements, it is generally true that the *trans*-isomers are more stable and have more marked absorption spectra with higher extinction coefficients (*5*).

The PADPCC was prepared by the reaction of *p*-phenylazodiphenylamine with phosgene. The material isolated and purified by crystallization from methanol had an absorption maximum at 332 nm with an extinction coefficient of 23,400. Exposure to ultraviolet light having a maximum intensity at 320 to 330 nm (Spectroline model B-100) caused disappearance of 332 nm peak and the appearance of a smaller shoulder at about 290 nm. Short exposure of the new compound to light of about 420 nm (*6*) resulted in the rapid reappearance of the original spectrum. From these data, we can assume that the more stable isomer—that is, the one originally isolated—is *trans* PADPCC. The *cis*-isomer could be isolated in pure form after exposure of the *trans*-isomer in cyclohexane to ultraviolet light (320 nm) followed by evaporation of the solvent in subdued light and chromatography on silica gel, with cyclohexane as the developing solvent. In this system, the two isomers migrated at different rates, the R_F for the *cis*-isomer being 0.04 and that for the *trans*-form being 0.18. The *cis*-isomer was stable in solution if kept in the dark; storage for as long as 2 days in the refrigerator caused no observable change in absorption spectrum. However, exposure to light of the wavelength about 420 nm caused rapid conversion to the more stable *trans*-isomer. The absorption spectra of the two isomers in methanol are shown in Fig. 1.

The reaction of the isomers with chymotrypsin was examined at 15°C in 0.05*M* tris(hydroxymethyl)amino methane-chloride buffer, *p*H 7.0, containing 0.5 percent methanol. The concentration of chymotrypsin was $1 \times 10^{-6}M$; that of the inactivators was $2 \times 10^{-6}M$. The second-order rate constants of inactivation were for the *cis*-form, $5300M^{-1}$ sec^{-1}, and for *trans*, $1150M^{-1}$ sec^{-1}. Thus, the *cis*-isomer was about five times more reactive than the *trans*-isomer.

Since the two isomers have different activities, and because their relative concentrations in solution can be controlled by light, it should be possible to use light to regulate the rate of inactivation of chymotrypsin (Fig. 2). *Cis-p*-azophenyldiphenylcarbamyl chloride (PADPCC) (1×10^{-6} mole/liter) was allowed to react with chymotrypsin (5×10^{-7} mole/liter) in 0.05*M tris*-chloride buffer (*p*H 7.0) containing 0.5 percent methanol, at 0°C in a darkened laboratory. Samples were withdrawn at suitable intervals and assayed for chymotrypsin activity (*2*). At 280 seconds,

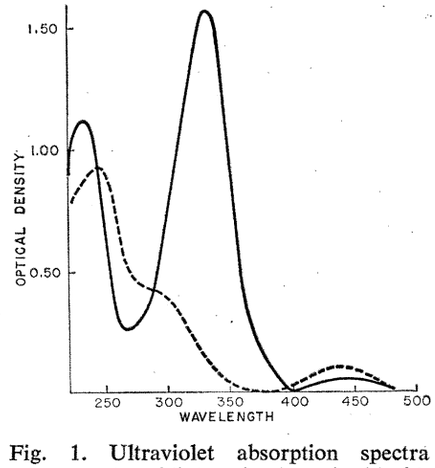

Fig. 1. Ultraviolet absorption spectra of *p*-azophenyldiphenylcarbamyl chloride (PADPCC) isomers, 6.31×10^{-5} mole/liter in methanol. *Trans*-isomer, solid line; *cis*-isomer, broken line.