tability indicate that no FEP Teflon remained on the polyethylene. If a vapor phase had been used instead of the FEP Teflon to generate the polyethylene surface, similar results would have been obtained except that the extent of surface roughness would have precluded examination by glancing-angle electron diffraction. Both wettability and infrared analysis experiments have indicated that the surface region of the polymer that crystallized when in contact with the gold was crystalline, whereas the specimen that solidified in contact with FEP Teflon remained noncrystalline.

Diffraction patterns were taken of the treated as well as untreated surfaces. In all cases the diffraction patterns obtained from the treated surfaces show several dense bands. These bands indicate definite changes in order-development of structure other than that of the bulk polymer. The diffraction patterns from untreated surfaces show no structure at all.

The calculated interplanar spacings, considering the broadness of the rings, at 4.20, 3.06, 2.5, and 2.26 Å, correspond quite closely to Bunn's (*5*) data for polyethylene, at 4.15, 3.72, 2.98, and 2.48 Å. From the diffraction data, the crystallite size is estimated to be 20 to 30 Å in diameter.

Glancing-angle electron diffraction confirms the earlier results obtained with wettability and infrared techniques. The nature of the interfacial region, with respect to crystallinity, is related to the substrate used to generate the polymer surface.

The glancing-angle electron diffraction not only furnished clear evidence of structural changes in the treated polyethylene film, but also proved that the substrates used in the formation of the film were removed completely. Thus, the increase in wettability of these films (*1*) is not due to traces of remaining substrate but is due solely to morphological changes in the surface structure of the polymer.

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Inhibition of Cell Growth in vitro by Adenosine 3′,5′-Monophosphate

Abstract. *Adenosine 3′,5′-monophosphate, at a concentration of 40 micrograms per milliliter, inhibits the growth of HeLa and strain L cells in culture. The inhibition becomes progressively greater during the incubation of the cells. Adenosine 5′-monophosphate and adenosine, metabolites of adenosine 3′,5′-monophosphate, do not affect the growth of either cell culture. This suggests that adenosine 3′,5′-monophosphate enters the cell without alteration. Dibutyryl-adenosine 3′,5′-monophosphate, reported to have a greater activity than adenosine 3′5′-monophosphate on several tissues, inhibited the growth of the cells much less.*

The action of steroids on cell cultures has not been extensively studied; however, they generally inhibit the growth of cells in culture (*1*). Hydrocortisone inhibits the growth of several human cell lines (*2*) and also inhibits the growth of chick heart fibroblasts and L cells (*3*). Arpels, Babcock, and Southam examined the effects of eight steroids on 12 human cell cultures, and cytotoxic effects were observed after incubation of the cell cultures for several days (*4*).

Adenosine 3′,5′-monophosphate (3′,5′-AMP) has been proposed as an intermediary or second messenger in the action of estrogens (*5*), epinephrine, glucagon, adrenocorticotrophin, luteotrophic hormone, and serotonin (*6*). Because the steroids inhibit growth of cell cultures, we determined whether 3′,5′-AMP would mimic this effect.

Strain L cells (NCTC clone 929) were maintained in Waymouth's medium MB752/1 (*7*), modified by replacing the salt solution with the tris (hydroxymethyl) amino methane–citrate salt solution described by Paul (*8*).

HeLa cells (line 229) were maintained in Eagle's medium (*9*). Both Eagle's and Waymouth's mediums were supplemented with 5 mg of neomycin sulfate, 0.2 mg of amphotericin B, and 10 ml of fetal calf serum per 100 ml of solution. The *p*H of the medium was adjusted to 7.6 at the beginning of all experiments.

L cells, obtained from 4-day-old stock cultures, were detached by scraping, and the HeLa cells were detached by treatment of the 4-day-old cultures with 0.1 percent trypsin (1 : 300, Baltimore Biological Laboratories). The cells were suspended by agitation, centrifuged, resuspended in fresh medium, and counted in a Coulter counter. Two milliliters of the medium containing 50×10^3 cells were pipetted into Leighton tubes.

Adenosine 3′,5′-monophosphate (Calbiochem, lots 73435 and 72982) and N^6-2′-*O*-dibutyryl-adenosine 3′,5′-monophosphate (Boehringer-Mannheim, lots 6068310 and 06477308) were dissolved directly in prepared culture medium and filtered through a prewashed Millipore filter.

The addition of 3′,5′-AMP (0.1 mg/ml) to the tissue culture medium inhibits its growth of L (Fig. 1) and HeLa cells. This inhibition becomes progressively more severe with time; the L cell count in the 3′,5′-AMP is 58 percent of the control at 2 days and 16 percent of the control at 4 days. The HeLa cell count is 55 percent of the control at 2 days and 27 percent of the control at 4 days. The addition of 5′-AMP to the cell cultures does not significantly inhibit growth. Adenosine was also added at the same molar concentration and it had no effect on the growth of the cell culture. The dose response to 3′,5′-AMP was investigated (Table 1), and at a concentration between 0.04 to 0.01 mg/ml the inhibition disappears.

To determine whether the growth retardation is the result of permanent cell damage or is reversible, we grew L cells in a Blake bottle in medium containing 3′,5′-AMP (0.1 mg/ml). After 4 days of incubation, the control cells and those in 3′,5′-AMP were counted. The cell count in the 3′,5′-AMP flask was only 14 percent of that in the control. The 3′,5′-AMP–treated cells were washed, suspended in fresh culture medium, and placed in Leighton tubes; they were then counted after incubation for 2 days and 4 days. Cell counts were compared to those for control cells not in 3′,5′-AMP. The 3′,5′-

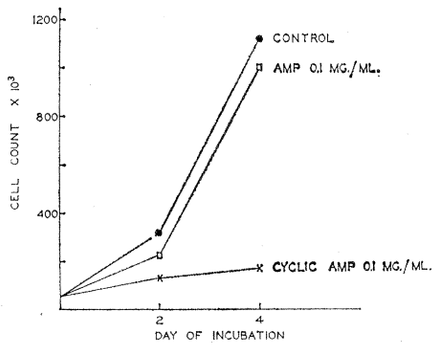

Fig. 1. Effect of 3′,5′-AMP and 5′-AMP on growth of strain L cells.

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