polysomes to ribonuclease and their sensitivity to trypsin is important because this may be significant for the stability of the polysomes in cells deprived of serum.

The argument could be advanced that only a small percentage of the total ribosomes are responsible for the protein synthesis observed. The dissociation of that minority would not be detected in the optical density pattern. However, 10^7 cells can make 200 μg of protein in 24 hours (Fig. 1A), which is about 40 percent of the total cell protein. The rate of amino acid incorporation corresponding to such net synthesis is reduced by at least 80 percent when serum is removed. Darnell *et al.* (*11*) conclude that at least 70 percent of the protein synthesis of Hela cells is carried on by polysomes. These disappear with a half-life of 3 hours in cells treated with actinomycin; protein synthesis by the cells decreases at the same rate.

The inactivating effect of serum deprival on ribosomal function may be related to observations with animal cells *in situ* and in culture reported by other investigators. Salb and Marcus (*12*) found that ribosomes from Hela cells in mitosis exhibit low activity and can be reactivated by trypsin. They proposed that basic proteins from the nucleus were the inhibitors. Monroy *et al.* (*13*) reported that trypsin reactivates ribosomes obtained from sea urchin eggs. Hoagland *et al.* (*14*) have presented evidence for the existence of an inhibitor of protein synthesis which is closely associated with polysomes prepared from rat liver.

The serum factor required for active polysomes could act directly on the protein synthesizing machinery or could neutralize a "governor" which normally regulates polysome function. The "governor" appears to inhibit polysome activity without dissociating the ribosomes from mRNA and without destroying mRNA.

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Enhanced Growth of Human Embryonic Cells Infected with Adenovirus 12

Abstract. *Fibroblast-like cells from human embryonic lung were infected with adenovirus type 12, and they survived as an established line, with the characteristics of "transformation" following considerable cellular killing. Inclusion bodies disappeared and cells became resistant to reinfection with type 12 virus as they grew in thick multilayered strands, and giant and syncytial cells became commonplace. An induced new cell antigen demonstrable by complement-fixation and fluorescent-antibody studies persisted for at least 20 culture passages after infection.*

After oncogenicity of human adenoviruses was observed in hamsters (*1*), we made attempts to "transform" (*2*) human cells with the oncogenic types of human adenoviruses. This became feasible when, in the course of developing a plaque assay with adenoviruses in human cells (*3*), a type 12 viral clone was isolated that appeared to be less lytic than other plaque-clones of the "wild" strain (Huie). Whether it is a mutant is being considered.

Human embryonic fibroblast-like lung (HEL) cells of the 21st passage in monolayer were infected with adenovirus at a multiplicity of about one. Other cultures of the same passage were not infected but were otherwise treated the same way. In 18 to 24 hours, inclusion bodies were seen in about 5 to 10 percent of cells that were stained with Giemsa. Cytopathogenic changes, typical of adenovirus infection, with enlargement of nuclei and eventual rounding of cells, developed within 3 to 4 days. The cytopathogenicity appeared less rapidly with type 12 than with types 4 or 7. Detachment and death of cells was practically complete in 10 to 14 days.

However, one of several adenovirus (type 12) plaques, when cloned and plated on fresh monolayers of HEL cells, appeared to be less lytic than other plaque-clones because a number of cells remained viable 10 days after infection. To support their survival, the augmented Eagle medium (*4*) was changed repeatedly at 3- to 4-day intervals. From these cells, two colonies of fibroblast-like cells began to grow out about 3 weeks after infection. Cells looked normal by phase microscopy, but typical adenovirus inclusions were visible in a few enlarged nuclei. The grown colonies were then trypsinized and transferred; uninfected control cultures that had not gone through the cytopathogenic stage of infection were treated in the same way. The latter grew less well, however, and failed to replicate after the 24th passage. On the other hand, the infected line of cells continued to multiply and have now been transferred altogether over 65 times since the original culture.

During repeated passage of adenovirus-infected cells, medium and cells together were checked periodically for the presence of virus by plating undiluted material (0.2 ml) on fresh monolayers of HEL cells. Figure 1 gives periodic titers of combined cell-associated and free virus and cumulative cell mass over a period of 24 weeks after infection. Virus was released steadily for at least eight passages and then the rate of release declined slowly during subsequent generations until cytopathogenic virus was no longer detectable at the 42nd overall passage. Such negative cultures were passed blindly on occasion in fresh HEL cells without issue. Giemsa staining and indirect immunofluorescent tagging of rabbit antiviral serum with fluorescein isothiocy-

anate dye confirmed these results. Although 1 to 5 percent of cells showed inclusions, these disappeared altogether 14 weeks after infection (the 33rd overall passage).

The population of cells after infection doubled about every 17 hours when growth space and medium were adequate. Even with as little as 2 percent fetal bovine serum they doubled in about 18 hours, in comparison with 25 hours for the uninfected HEL cells under optimum conditions. Several nonproducing cells of the infected line, growing under 0.3 percent agar medium (*5*), started to divide on the third day after seeding and grew out as discrete colonies when a minimum of 10^4 cells in 5 ml were incubated. No colonies grew when 10^3 cells were seeded. Uninfected HEL cells, although seeded with as many as 10^6 cells, failed to form colonies. There was little or no change in the appearance of cells after infection, except for inclusions, until about the 12th passage (80 days), at which time giant and syncytial cells began to appear commonly in addition to fibroblast-like cells (see Fig. 2); but they occurred rarely in the uninfected HEL cultures. Also, many of the cells became polygonal, and nuclei were often enlarged.

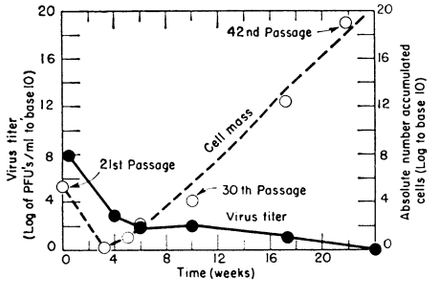

Fig. 1. Adenovirus type 12 titers and cell mass (total cell population) after infection of HEL cells.

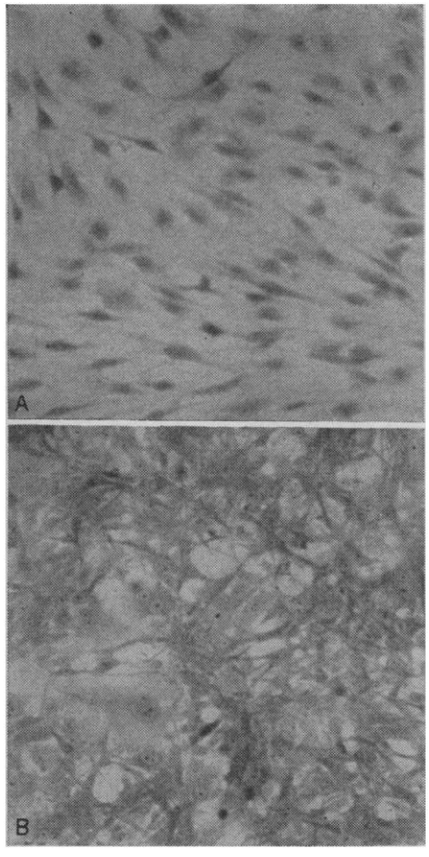

Fig. 2. (A) Monolayer of normal human embryonic lung fibroblastic cells in 19th passage. (B) Piling up in monolayer of human embryonic lung cells showing giant and syncytial cells in 40th passage following infection with adenovirus 12. A and B: Giemsa stain: × 180.

Following the 12th passage after infection, cells developed increasing resistance to reinfection with adenovirus type 12, as determined by Giemsa staining and plaque-forming units. Inclusion bodies were present in at least 40 percent of control HEL cells 48 hours after infection with types 4, 7, and 12, whereas in the 17th passage after infection (with type 12) they appeared in over 75 percent of cells secondarily infected with type 4, rarely with type 7, and not at all with type 12. Also, 72 hours after infection of control cultures, viral growth of types 7 and 12 increased 10- to 100-fold, but titers failed to rise after secondary infection of HEL cells in their 19th passage after infection with these types. There was, however, a 10-fold rise after infection with type 4 virus. Resistance to type 7 increased greatly but not, as yet, to type 4.

Table 1 gives results of complement-fixation tests: a mixture of whole and disrupted HEL cells, after infection as well as uninfected, was used as antigens. The former had a titer of 1 : 10 against four units of serum antibody from hamsters bearing tumors induced by adenovirus, either type 12 or type 7, and of less than 1 : 5 against normal hamster serum (*6*). Complement-fixation was confirmed by immunofluorescence with serum from tumor-bearing hamsters. About 40 percent of the cells of the 19th passage after infection showed specific fluorescence with tumor-bearing hamster serum. Fluorescent speckling of the previously infected HEL cells was limited to the cytoplasms, as noted by Pope and Rowe (*7*) in hamster cells "transformed" by adenovirus. Intranuclear fluorescence described in hamster and in human cells acutely infected with adenovirus (*7–9*) was not seen in the HEL cells. New cell antigen was not detected by complement-fixation with serum from hamsters bearing SV40 tumors.

Table 1. Complement-fixation with HEL cells, after infection and control, and serum obtained from tumor-bearing and normal hamsters. CAI, cells after infection; UC, uninfected cells; CF, complement-fixation. CF titer is expressed as reciprocal of highest dilution of antigen giving at least +++ reaction in complement fixation. "After-infection" cells were in their 40th passage; uninfected cells, in their 17th passage.

Antigen (10% suspension)	Antibody (hamster serum)	CF titer
CAI	Tumor-bearing (Adeno 12)	10
CAI	Tumor-bearing (Adeno 12)*	10
CAI	Tumor-bearing (Adeno 7)	10
CAI	Tumor-bearing (SV40)*	< 5
CAI	Normal	< 5
UC	Tumor-bearing (Adeno 12)	< 5
UC	Tumor-bearing (Adeno 7)	< 5
UC	Normal	< 5

* Obtained from Flow Laboratories, Rockville, Maryland.

There is no doubt that several characteristics of the HEL cells changed after infection, but whether the "transformation" was neoplastic cannot be proved because of the impracticability of implanting human cells into a histocompatible host. Neither has it been proved that all changes were the immediate results of infection. Nevertheless, the decrease in doubling-time of the population, the morphological changes of cells and of growth characteristics, the acquisition of resistance to reinfection, the reduction and apparent cessation of virus-release, the appearance of a new antigen, and the active growth in semisolid medium, all favor the phenomenon called "transformation." That the changes were not spontaneous is favored both by persistence of the new cell antigen and by cellular resistance to reinfection with adenovirus type 12. An equivalent, spontaneously transformed human embryonic lung cell line (*10*) maintained in our laboratory for over 5 years continues to be susceptible to adenovirus types 4, 7, and 12.

The "transformation" reported here favors the concept that it was induced by the human source virus and sug-

gests that it may occur in man under certain undefined conditions. However, at this time there is no evidence that adenovirus infections have been associated with tumors in man.

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Anteroventral Cochlear Nucleus: Wave Forms of Extracellularly Recorded Spike Potentials

Abstract. *Analysis of wave forms of spike potentials from neurons in the anteroventral cochlear nucleus indicates that the spikes are composed of three components. Two appear to be postsynaptic events, and one appears to be presynaptic and to be related to the calyces of Held found in that part of the nucleus.*

Spike potentials recorded extracellularly from neurons in the most rostral region, or "oral pole," of the anteroventral cochlear nucleus (AVCN) have a wave form that is not found in recordings from other regions of the cochlear nucleus. The occurrence of these wave forms in this region appears to be related to the anatomical finding that the AVCN contains large nerve terminals, called calyces (or bulbs) of Held, that are not found in other regions of the nucleus (*1*).

Adult cats were anesthetized (Dial in urethane, 0.75 mg/kg), and the cochlear nucleus was exposed by partial aspiration of the cerebellum. Spike potentials were recorded with indium-filled, platinum-black-tipped microelectrodes with tip diameters from 4 to 12 μ (*2*); the reference electrode was connected to the head-holder. This report is based on observations and recordings of spike potentials of both spontaneous and acoustically stimulated activity from several hundred neurons in the AVCN of more than 25 cats (*3*).

Superposed traces of spike potentials for six different neurons are shown in Fig. 1. The samples shown are representative of the variations in wave forms encountered. The prominent property that distinguishes these spike potentials from those encountered in other regions of the cochlear nucleus is the presence of a positive (P) component early in their wave form. The P components are all-or-nothing in character (that is, for a given neuron, their amplitude is independent of stimulus level); they are usually either monophasic or diphasic (+, −); they each are consistently followed by a prominent negative potential (*4*). The negative potentials, alone, are characteristic of those generally obtained with extracellular recordings of spike discharges of neurons (*5*).

Let us first consider the negative potentials. The inflection on the rising phase of the negative potentials of several of the samples shown suggests that they consist of two components (A and B) that are similar to the composition of spike potentials recorded from spinal motor neurons and elsewhere (*5–7*). For some neurons (about 20 to 25 percent of our sample) the two-component structure is explicit because the second, B, component occasionally fails to develop (Fig. 2). This two-component structure has been distinctly observed, thus far, only for neurons in the "oral pole" of the AVCN, although inflections on leading edges of spike potentials have been observed elsewhere in the cochlear nucleus.

Factors affecting the development of the A and B components of spike potentials have been studied extensively by intracellular techniques in spinal motor neurons (*6*) and cortical neurons (*7*). The A component has been identified as the discharge of the initial segment (IS) or axon hillock of the neuron, and the B component has been identified as the discharge of the soma-dendritic process (SD) (*5*).

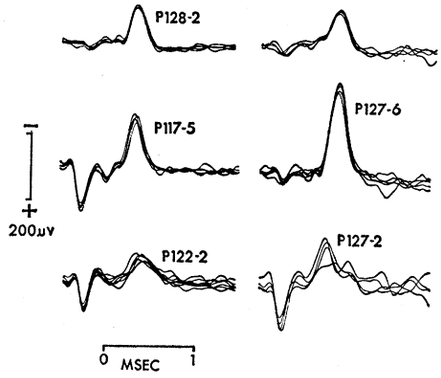

Fig. 1. Superposed traces of spike potentials of six different neurons in the "oral pole" of the anteroventral cochlear nucleus. These illustrate the variety of wave shapes obtained and the consistent appearance of a positive (P) component. These traces were not synchronized to any stimulus but were synchronized to an arbitrary point on the wave form after they had been recorded from the neuron.

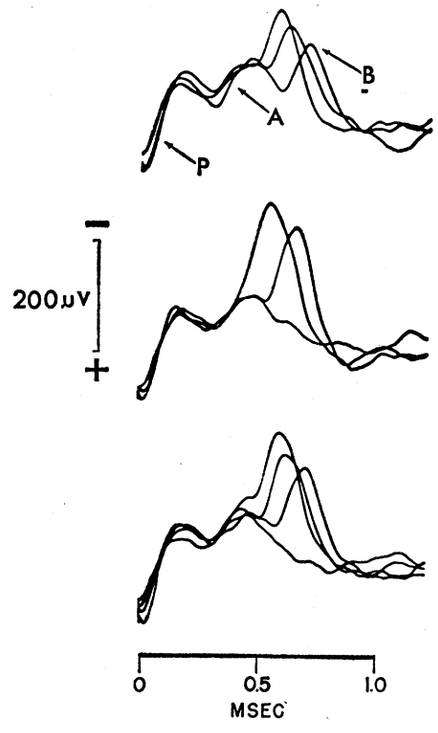

Fig. 2. Superposed traces of spike discharges from a single neuron which illustrate the two-component structure of the negative potential. These spike potentials were recorded in response to a continuous sinusoidal acoustic signal delivered to the ear. In general, the inflection on the rising phase increases in size as the time from previous spike discharge decreases (not shown). The fact that explicit two-component structure is seen for only 20 to 25 percent of the neurons may be related to electrode location relative to the neuron. These traces were synchronized to a point on the leading edge of the P component.