calcium ions were applied at *p*H 8.7 (*8*). These facts strongly support Worley's hypothesis that contractile systems concerned with beat and with reversal may be separable and distinct from each other (*9*).

In another series of experiments, a glass microcapillary electrode (about 0.5 μ in tip diameter) filled with 0.1*M* NH_4Cl (*10*) was introduced into cytoplasm of *Paramecium*, and the potential difference between the inserted electrode and a reference electrode placed in the external solution was amplified, displayed on a cathode-ray tube, and photographed.

Penetration of the microelectrode into cytoplasm of a normal *Paramecium* was generally accompanied by a sudden shift to a negative direct current level in recorded potential ranging from −15 to −40 mvolt (*10, 13*). Sometimes small, irregular, repetitive fluctuations in potential level (less than 1 mvolt) were found (Fig 2A). Intracellular potentials of a similar nature were also recorded from a nickle-treated *Paramecium* with nonbeating cilia (Fig. 2B). Therefore, the small perturbations in potential level would appear to have no relation to ciliary beat. Moreover, the degree of the inside negativity in nickel-treated *Paramecium* (21 ± 1.0 mvolt; mean and S.E. of five measurements on five different specimens) was found to be almost equal to that in normal *Paramecium* (20 ± 0.27 mvolt; mean and S.E. of five measurements on five different specimens). In contrast, in the ciliated cells of the ctenophore comb plate, depolarizing action potentials are associated not only with natural ciliary beats but also with beats initiated in response to mechanical vibrations of cilia (*11*).

Low concentrations of extracellular calcium (*12*) frequently cause spontaneous reversal in the ciliary beat of normal specimens (*12*). Similar spontaneous reversals of orientation occur in nickel-treated specimens in media with a low calcium concentration as does nickel-treated *Paramecium* without ciliary beat. Spontaneous reversals in normal specimens are accompanied by transient membrane deplorizations (*10, 13*). Recordings of intracellular potentials of nickel-treated *Paramecium* made simultaneously with photographic recording of spontaneous reversal responses of nonbeating cilia in 0.01 m*M* $CaCl_2$ (Fig. 3) show that the reversal response of nonbeating cilia are always associated with depolarizations of the membrane.

It is concluded that membrane depolarization is specifically concerned with the ciliary reversal and not with ciliary beat in *Paramecium*.

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Arrested Protein Synthesis in Polysomes of Cultured Chick Embryo Cells

Abstract. *Cells deprived of serum synthesize proteins at a reduced rate; when serum is restored the rate returns to normal. The polysomes do not dissociate, but show reduced incorporation of amino acid in vitro, and are less responsive to polyuridylic acid than are those from normal cells.*

In primary cultures of chick embryo cells, protein synthesis shows an absolute requirement for serum (*1*): in its absence there is no detectable net synthesis of protein or RNA, and incorporation of precursors into these classes of macromolecules is sharply curtailed. The three principal classes of RNA—ribosomal, messenger, and soluble—continue to be synthesized but at a very reduced rate (*2*).

In order to identify the phase of protein synthesis that requires serum, the effect of serum withdrawal from cells that are synthesizing protein at the maximum rate was explored. The results suggest (i) that polysomes remain intact when deprived of serum; (ii) that messenger RNA (mRNA) is not destroyed; and (iii) that these polysomes are relatively ineffective in incorporating amino acids in vitro.

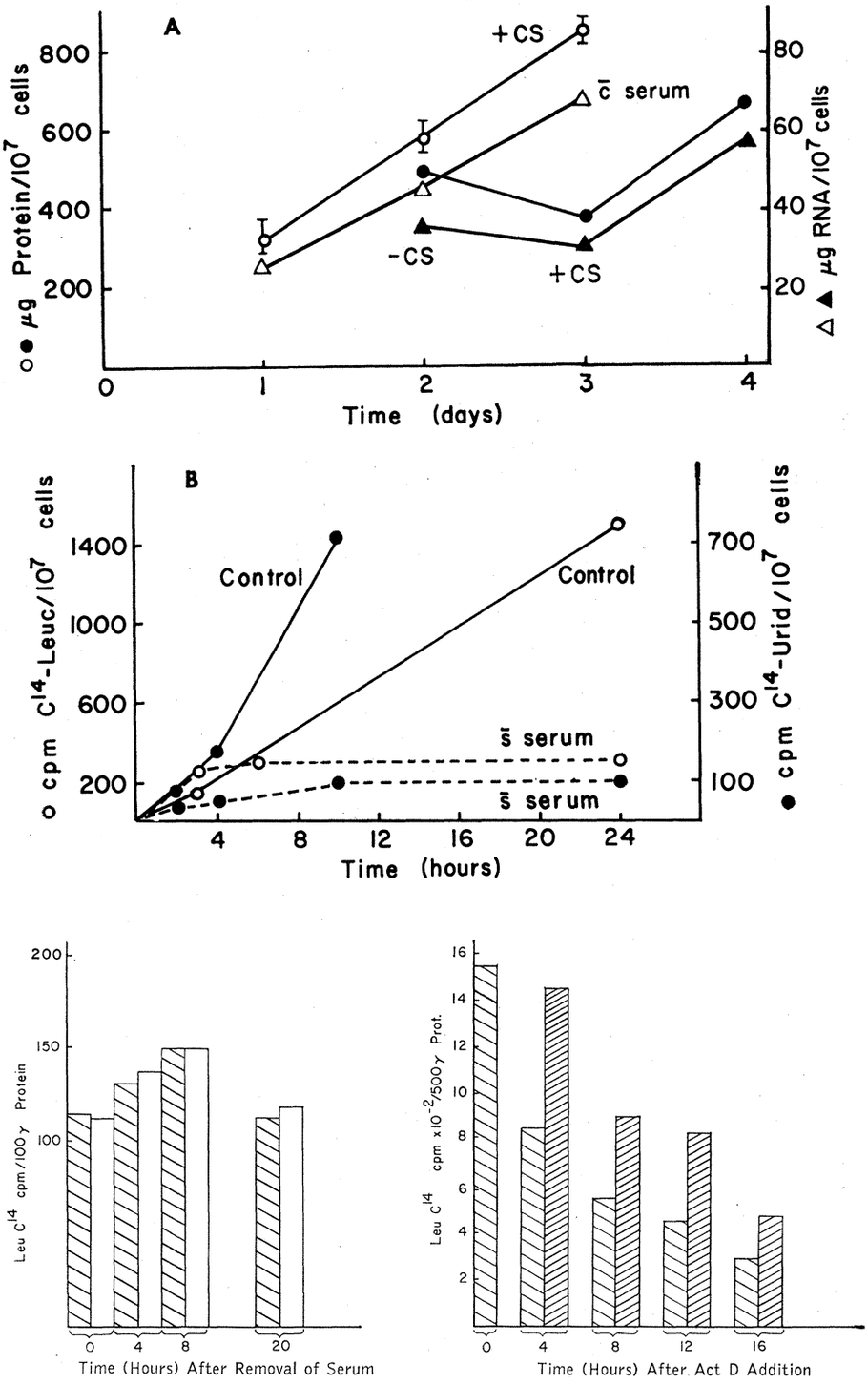
Cells cultured as monolayers in Eagle basal medium (*3*) with 3 percent calf serum synthesize protein for several days, the rate of growth being linear (*1*). When the medium was replaced by one without serum at any time during the linear phase of growth, the net protein and RNA syntheses ceased abruptly (Fig. 1). The amount of cellular protein and RNA decreased during the next 24 hours by approximately 20 percent, probably representing cells which had become detached from the surface.

When serum was added to the cells at any time for several days after its initial removal, the rate of protein synthesis increased rapidly (Figs. 1A, and 2), and within 4 hours the rate was equal to that of cells maintained in serum. An unusually high initial rate of protein synthesis is often observed under such circumstances (*4*). For 24 hours after serum had been restored, the net amount of protein synthesized was essentially normal.

When actinomycin D was added with the serum, after the cells had been deprived of serum for various periods, the initial rate of protein synthesis (that during the first 4 hours) (Fig. 2), was not adversely affected. It thus appears that when serum is absent mRNA is present but not translated.

These results could be due to persistence or to renewal of mRNA while the cells were deprived of serum. To distinguish between these possibilities, actinomycin was used to prevent renewal of mRNA during incubation without serum. The capacity for protein synthesis was then tested after the serum was restored. This capacity slowly decreases during 16 hours (Fig. 3), but the decrease was even faster in identically treated cultures incubated with both actinomycin and serum. It appears that depriving cells of serum impairs the translation of mRNA but does not impair, and even promotes, the persistence of RNA in functional form. Although the effect of actinomy-

Fig. 1 (right). Effect of serum removal on protein and RNA synthesis. (A) Cells cultured in Eagle basal medium with 3 percent calf serum for 48 hours were washed twice with balanced salt solution. Fresh medium with 3 percent serum was added to half the bottles and the medium without serum to the other half. (*CS*, 3 percent calf serum). Cells were harvested and proteins were estimated by the Lowry procedure (*1*). Each point represents the average of two bottles of approximately 10^7 cells per bottle. (B) Incorporation of C^{14}-leucine (0.25 μc per bottle) or C^{14}-uridine (0.25 μc per bottle) into the fraction insoluble in cold trichloroacetic acid. Labeled precursors were added when the medium was replaced. Estimate of cumulative amino acid incorporation was made by harvesting the cells from two bottles for each point indicated.



cin D on the synthesis of RNA is probably its primary effect, secondary effects cannot be dismissed (*5*). Since its addition did not hinder the recovery of protein synthesis, secondary toxic effects need not be considered in interpreting these results.

The effect of serum deprival upon the synthesis of protein in the whole cell might be the result of altered ribosomes or of soluble factors associated with protein synthesis.

Microsomes were obtained from cells grown in pyrex dishes (surface area approximately 540 cm^2). The cells were rinsed with Hanks' balanced salt solution, scraped from the glass surface with a rubber spatula, and chilled. Next they were centrifuged and washed, first with cold saline and then quickly with hypotonic standard buffer for reticulocytes (RSB) (*6*). The cells were suspended in RSB on ice and allowed to swell for 30 to 45 minutes, after which they were disrupted by grinding with a Dounce glass homogenizer. The extract was centrifuged at 15,000*g* for 10 minutes in a refrigerated Servall to remove nuclei and mitochondria. The microsomes were obtained by centrifuging the supernatant for 2 hours through 1.0*M* sucrose (*p*H 7.5) at 100,000*g* in a Spinco preparative ultracentrifuge at 4°C. Microsomes largely free of endoplasmic reticulum were also obtained by deoxycholate (0.5 percent) treatment of the S-15 supernatant fluid (*7*). The fluid was then layered over a medium of 0.25*M* sucrose and centrifuged for 2 hours at 40,000 rev/min. The pellet obtained was resuspended in RSB for use in the studies of incorporation of amino acids in vitro.

Microsomes, whether or not treated

Fig. 2 (left). Effect of actinomycin D on reactivation of protein synthesis by restoring serum. The serum was removed at time zero from the medium after 48 hours by the procedure followed in Fig. 1. At various times thereafter serum was restored to the medium with or without actinomycin D (1.0 μg/ml) and with C^{14}-leucine. After 4 hours the cells were harvested and the incorporation of C^{14}-leucine into TCA-insoluble material was measured. The incorporation of C^{14}-uridine into cells treated with actinomycin D was less than 8 percent of the control value (first 4 hours after actinomycin D was added). Hatched bar, control—serum restored; unhatched bar, actinomycin D when serum restored. Fig. 3 (right). Effect of serum deprival on persistence of capacity for protein synthesis. Actinomycin D (0.5 μg/ml) was added at time zero to cultures freshly transferred to medium with or without serum. After prior incubation for varying intervals serum was added to the cultures lacking serum, and the amount of C^{14}-leucine incorporated into TCA-insoluble material in the subsequent 4 hours was determined in both cultures. C^{14}-uridine incorporation into cultures treated with actinomycin D was less than 8 percent of control (first 4 hours after actinomycin D was added). Wide hatches, control; narrow hatches, serum-deprived.

with deoxycholate, incorporated amino acids in vitro; incorporation was stimulated by the addition of polyuridylic acid (polyU) (Fig. 4A). Microsomes from cells treated with actinomycin D had a reduced capacity for the endogenous incorporation of amino acids, but were stimulated to a greater degree by polyU.

Microsomes obtained from cells deprived of serum for 24 hours had reduced incorporating activity per unit of endogenous RNA. Moreover they responded only slightly when polyU was added (Fig. 4B). When serum was restored, the microsomes returned to full activity within 3 hours (*8*). The addition of serum or dialyzed serum to the in vitro system, however, did not alter the activity of the microsomes from cells either provided with or deprived of serum. If we assume that the microsomes treated with deoxycholate are substantially free of membrane, the ribosomes are probably inactivated by the withdrawal of serum.

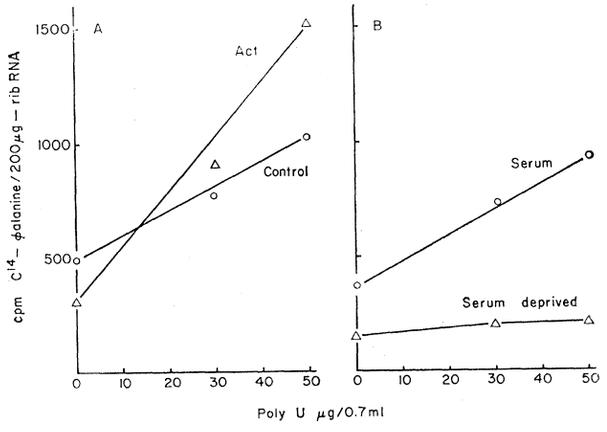
To compare the optical density profiles of the polysomes of normal cells and of cells deprived of serum, the pellet obtained from deoxycholate-treated microsomes after they were centrifuged through 0.25*M* sucrose at 40,000 rev/min was recentrifuged through a sucrose gradient (15 to 30 percent, weight/volume) in RSB at 35,000 rev/min (Spinco rotor SW39) for 1.5 hours at 4°C. Optical density at 260 mμ was determined with a Gilford flow cell and an automatic recording device. Fractions were collected arbitrarily from the gradient and ribosomes were removed from them for the incorporation in vitro.

The removal of serum for as long as 24 hours did not modify in any significant way the optical density profile (Fig. 5). Incorporation of amino acids by endogenous RNA in fractions obtained from the gradient was, however, markedly reduced in the preparations from cells deprived of serum. In both preparations amino acid incorporation occurred mainly in the larger polysomes. The use of a normal supernatant fraction in these incubations may have minimized the differences by providing elements in short supply in ribosomes prepared from cells deprived of serum.

Several tentative conclusions are drawn. (i) In the cultivated chick cell a class of polysome exists that is resistant to ribonuclease and sensitive to tryspin. (ii) In the absence of serum some of these polysome complexes remain intact but cease functioning. (iii) Their failure to function is not due to mRNA destruction; it persists when polysomes released from cells are tested in vitro. (iv) Their insensitivity to polyU stimulation in vitro suggests either the absence of a required polysomal factor or the presence of an inhibitor. (v) Serum contains an "activator" which itself restores polysome function in cells or induces the synthesis of such a molecule without synthesis of new RNA; the "activator" is not effective in vitro. (vi) The actinomycin D–induced decrease of the cell's capacity to synthesize protein is curtailed in the absence of serum.

We propose to call the aggregates of inactivated ribosomes "arrested polysomes" although they apparently differ qualitatively from those to which the term polysome was originally applied. They are relatively resistant to ribonuclease (2 μg/ml for 30 minutes at 2°C), but they dissociate to form single ribosomes when exposed to trypsin (10 μg/ml; plus ribonuclease, 2 μg/ml at 2°C). Similar responses to enzymatic treatment have been described by Rabinowitz *et al.* (*9*) for polysomes from the heart muscle of chick embryos; Humphries *et al.* (*10*) have observed polysomes resistant to ribonuclease from dermal cells of chick embryos.

The fact that polysomes from the uncultured 10-day chick embryo are sensitive to ribonuclease (1 μg/ml for 30 minutes at 2°C) suggests that in cell culture certain cell types may be selected, or particular species of polysomes formed. The resistance of the



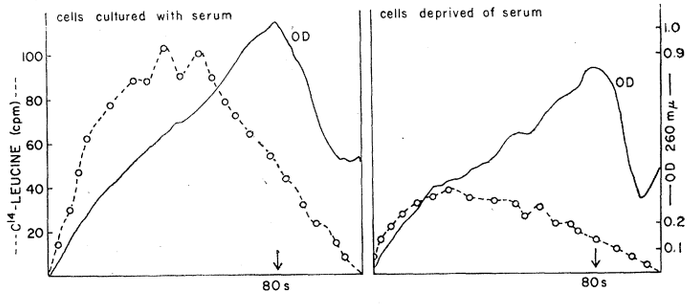


Fig. 4 (left). Amino acid incorporation by deoxycholate-treated microsomes from cells grown with actinomycin D or without serum. Cells were disrupted in a Potter-Elvehjem homogenizer and the microsomes were collected; the homogenate was cleared of debris, treated with deoxycholate, then centrifuged at 100,000*g*, the microsomes being collected in the pellet. Microsomes from cells exposed to actinomycin D (0.1 μg/ml) for 16 hours or cells deprived of serum for 24 hours were compared with microsomes from untreated cells in the presence of the supernatant fraction prepared from normal chick embryo cells. The cells were incubated for 40 minutes at 37°C. Total volume was 0.7 ml: mercaptoethanol, .006*M*; ATP, $0.9 \times 10^{-3}M$; creatine phosphokinase, 28 μg; creatine phosphate, 1.0 mg; GTP, $10^{-4}M$; KCl, $7.6 \times 10^{-3}M$; MgAc, $5 \times 10^{-3}M$; C^{14}-phenylalanine, 0.25 μc; 19 amino acids, 0.025μ*M* in each; approximately 1.5 mg of S-100 protein (*7*) and 100 to 200 μg of microsomal RNA; polyU (Miles) as indicated. *Act*, actinomycin D. Fig. 5 (right). Amino acid incorporation into polysomes of normal cells and cells deprived of serum. Microsomes from cells cultured in 3 percent serum and from cells of the same population deprived of serum for 24 hours were treated with sodium deoxycholate (0.5 percent) and subjected to sucrose gradient fractionation at 4°C. Fractions from the gradient were used as ribosomes for studies of in vitro amino acid incorporation due to endogenous RNA; a normal supernatant fraction from whole chick embryos was used. Conditions for incorporation are the same as those in Fig. 4.

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-