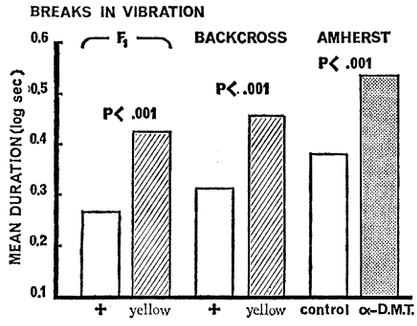

Fig. 3. Mean duration of breaks in the wing vibration component during the first 2 minutes of male courtship with single pair matings with wild-type females.

sine provides a common precursor for synthesis of melanin pigment and of catecholamines, such as dopamine and noradrenalin, which play an important role in the functioning of the nervous system (*8*). The action of α-DMT in mimicking the effects of the mutant may be an indication that the *yellow* gene is involved in some step common to both of these biosynthetic pathways.

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Cytomegalovirus: Conversion of Nonpermissive Cells to a Permissive State for Virus Replication

Abstract. *Human embryonic kidney cells are epithelioid cells which are normally nonpermissive for in vitro replication of human cytomegalovirus. These cells were converted to a permissive state for the virus by prior treatment with 5-iodo-2′-deoxyuridine. When this method was used, a nonpermissive cell was made permissive to an infecting virus.*

Human cytomegalovirus (CMV) is a member of the herpesvirus group (*1*). It is host specific and replication in vitro is limited to human fibroblasts (*2*). This specificity in vitro for fibroblastic cells is puzzling because the virus is often isolated from epithelioid cells taken from the host (*3*).

Certain thymidine analogs, such as 5-iodo-2′-deoxyuridine (IdU) and 5-bromodeoxyuridine, can induce cells to produce C type virus particles (*4*). They can also induce Epstein-Barr virus–negative (nonproducing) lymphoblastoid cells and somatic cell hybrids produced with these lymphoblastoid cells to produce Epstein-Barr virus, a member of the herpesvirus group (*5, 6*). These same analogs can also depress the synthesis of certain cell proteins such as interferon (*7*). We have reported that preliminary treatment of human fibroblast cells with IdU enhances the replication of CMV in these cells (*8*). We have now investigated the possibility that prior treatment with IdU might enable human CMV to replicate in nonpermissive epithelioid cells.

The epithelioid cells used in these studies were human embryonic kidney cells (HEM Laboratories, Rockville, Maryland). Second-passage confluent monolayers were grown in medium 199, supplemented with 10 percent tryptose phosphate broth, 10 percent fetal calf serum, 100 units of penicillin, 100 μg of streptomycin, and 0.075 percent sodium bicarbonate. The cells were dispersed with trypsin and centrifuged; the sedimented cells were resuspended to the desired concentration. The thymidine analog IdU (100 μg/ml) was added, and the cells were then placed in appropriate containers. The cells were incubated for 96 hours in the presence of IdU, washed two times with isotonic tris buffer, *p*H 7.4, and then exposed to either virus or growth medium containing the same concentration of fetal calf serum as the virus inoculum. Control cultures were treated in the same manner except that IdU was not added. The AD-169 strain of CMV was used throughout our studies. We detected virus DNA by labeling the DNA of infected and sham-infected cultures with tritiated thymidine ([^{3}H]dT) (17 c/mmole; Schwarz/Mann), separating cellular and virus DNA by isopycnic centrifugation in CsCl, and determining the amount of [^{3}H]dT incorporated into acid-insoluble material (*9*).

We first attempted to determine whether CMV DNA was produced in epithelioid cells. The HEK cells with IdU, both treated and untreated, were grown in 1-ounce (1 ounce about 30 ml) glass prescription bottles. The cultures were either infected with 1 plaque-forming unit (PFU) of CMV per cell or were sham-infected with medium. The cells were then grown in the presence of [^{3}H]dT (10 μc/ml) for 24-hour periods. The amount of [^{3}H]dT incorporated into acid-insoluble material was then determined (*9*). Figure 1 represents the cells exposed to [^{3}H]dT for 48 to 72 hours after inoculation with CMV. Figure 1A represents the cells not treated with IdU but infected with CMV. All DNA that contained [^{3}H]dT in these cultures banded at a density corresponding to that of reported values for cellular DNA (*9*). No evidence was found during the entire period of this experiment (0 to 120

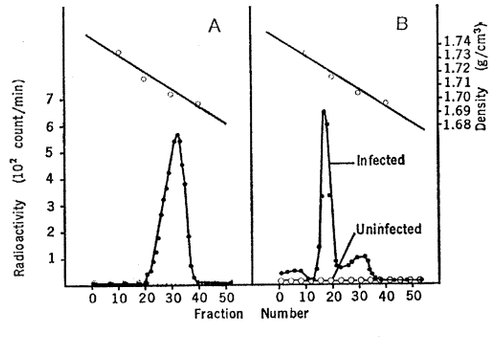

Fig. 1. DNA synthesis in cultures pretreated with 5-iodo-2′-deoxyuridine or untreated and inoculated with human cytomegalovirus strain AD-169 or growth medium containing the same serum concentration as the virus inoculum. The cells were treated with IdU (100 μg/ml) and then inoculated as described in Table 1. They were then treated for 24-hour periods with [H^3]thymidine (10 μc/ml; specific activity, 17 c/mmole). This figure represents the 48- to 72-hour treatment. The closed circles represent infected cultures, and the open circles represent the sham-infected cultures. The analysis of DNA has been reported (*9*). Cellular and virus DNA were separated by isopycnic banding in CsCl. DNA was precipitated in 5 percent trichloroacetic acid, and the incorporated [^{3}H]dT in the acid-precipitable material was determined (counts per minute) in a liquid scintillation counter.

hours) of CMV DNA in the untreated cells. Figure 1B represents the DNA isolated from cells that had been treated with IdU. There were three peaks from the gradient containing infected cells: a normal cell DNA peak in fraction 32, a peak consisting of cell DNA which contains IdU peak in fraction 4 and a DNA peak in fraction 17 with a density (1.718 g/cm^3) similar to that reported for human CMV DNA (*10*). The CMV peak in fraction 17 cosedimented with CMV marker DNA; the DNA peak in fraction 32 cosedimented with mammalian cell marker DNA. The density shift of the cellular DNA substituted with IdU (fraction 4) has been reported (*11*). Our results indicate that prior treatment of epithelioid cells with IdU allows replication of CMV DNA.

The data in Fig. 1B also indicate that prior treatment of cells with IdU reduces cellular DNA synthesis (as measured by [^{3}H]dT uptake) to a negligible level (open circles). However, in the infected cells, there is an increase in [^{3}H]dT uptake into cellular DNA, suggesting possible stimulation of host DNA synthesis by the virus.

We next attempted to determine whether infectious CMV was produced in HEK cells that had been first treated with IdU and then infected with CMV. HEK cells were grown in 1-ounce glass prescription bottles either in the presence or absence of IdU (100 μg/ml) as described above. After the cultures had been in the presence of IdU for 96 hours, they were rinsed twice with isotonic tris buffer (pH 7.4) and then infected with CMV (1 PFU/cell). The virus was adsorbed for 1 hour and then maintenance medium was added. Cultures were harvested periodically after virus inoculation. We determined by the plaque assay (*8*) the amount of infectious virus produced. The control cells were incubated in the same manner except that no IdU was added. They produced no detectable virus during the 7-day period of the study. The cells treated with IdU, however, produced more than 3 log units of infectious virus (Table 1). Cytopathology was observed in drug-treated infected cultures but less CMV was produced than that reported for permissive cells (*8*). Nevertheless, the experiment unequivocally shows that infectious CMV was produced in cells which are nonpermissive to infection in vitro with human CMV (*3*).

Our studies demonstrate that human CMV can replicate in epithelioid cells. In view of what is known about the action of IdU, it seems possible that a cellular product produced in epithelioid cells which normally interferes with replication of CMV in these cells is depressed, thus allowing virus replication to occur. The data presented (Fig. 1B) indicate that IdU treatment of cells greatly reduces cellular DNA synthesis. This effect may play an important role in the conversion of nonpermissive cells to cells which are permissive for viral replication.

Table 1. Replication of human cytomegalovirus in epithelioid cells. Three plates per dilution of sample were used for each determination.

Time after inoculation (days)	Virus titer (PFU/ml)	
	Untreated	IdU-treated
2	<2	3.3
3	<2	365
4	<2	600
5	<2	2000
6	<2	850
7	<2	950

The technique of prior treatment of cells with IdU in order to convert nonpermissive cells to a state of permissiveness or to enhance replication in permissive cells (*8*) may hold a great deal of promise in studying virus agents that have proved difficult to replicate in vitro (for example, cancer viruses and infectious and serum hepatitis viruses). The technique should also prove useful in investigating the factors that render cells nonpermissive to virus infection.

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Catecholamine and Dibutyryl Cyclic AMP Effects on Myosin Adenosine Triphosphatase in Cultured Rat Heart Cells

Abstract. *Catecholamines and dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP) increase the activity of myosin adenosine triphosphatase in cultured rat heart cells. Dichloroisoproterenol, an inhibitor of the beta receptor of the catecholamines, inhibits the action of the catecholamines but not of cyclic AMP.*

We have reported that myosin in rat heart cells in culture is maintained if serum is present in the medium (*1*); serum acts both by stimulation of myosin synthesis and by protection against its degradation (*2*). A heat-stable fraction (HSF) separated from the serum (*3*) stimulates myosin synthesis but has little effect on myosin degradation (*4*). Specificity is indicated by the observation that HSF has little effect on lactic dehydrogenase or creatine phosphokinase in cultured rat heart cells or on myosin in cultured cells of rat skeletal muscle (*3*). Also HSF stimulation of uptake of radioactive amino acid into myosin is two to five times greater than stimulation of uptake into average heart protein (*4*). The small size, heat stability, and relative specificity of the factor have led us to suspect that it might be hormonal and that adenosine 3′,5′-monophosphate (cyclic AMP) would be active. We report here that cyclic AMP and the catecholamines significantly increase myosin enzyme activity in cultured rat heart cells.

Rat heart cells were plated and incubated in complete medium (*5, 6*) for up to 4 days and shifted to minimal medium (medium without serum) to which catecholamines or cyclic AMP had been added. At all concentrations tested (Table 1), cyclic AMP and the catecholamines increased the adenosine triphosphatase activity by nearly 100 to 400 percent. There is no growth or division in cells incubated without