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

Table 1. Effect of cyclic AMP and catecholamines on activity of myosin adenosine triphosphatase. Heart cells from rats 2 to 4 days old were cultured (*5*) in CMRL-1415-ATM medium (*6*) with 10 percent fetal calf serum and 10 percent horse serum plus antibiotics. Cells (5×10^6) were incubated in 20 by 100 mm plastic petri dishes for 2 to 4 days in complete CMRL medium before being transferred to the experimental medium. The first value represents the activity on the complete medium just before the change. The numbers in parentheses represent the days of incubation. Adenosine triphosphatase activity was measured as reported (*2*) and represents the change in inorganic phosphate per minute per milligram of total cellular protein. The activity as measured is equivalent to myosin content. The activity is extractable and soluble in high salt solution, precipitable by dilution, activated by calcium, and inhibited by magnesium. Further, myosin has been extracted and separated and its concentrations measured directly; and in all cases tested the myosin content is directly correlated to the enzyme activity measured in the crude extract.

Concentration (M)	Myosin adenosine triphosphatase (units per milligram of total protein)		
	Before transfer	After transfer	
	Dibutyryl cyclic AMP		
0	29 (2)	48.9 (4)	
10^{-5}		88.6	
10^{-4}		100	
5×10^{-4}		106	
	Epinephrine		
0	138 (4)	24.5 (5)	26.2 (7)
10^{-6}		70.4	70.9
10^{-4}		67.3	18.9
	Isoproterenol		
0	133 (3)	27.7 (5)	60 (7)
10^{-7}		47.1	97
10^{-6}		87.4	113
10^{-5}		62.4	171
10^{-4}		114	90.4
	Norepinephrine		
0	133 (3)	27.7 (5)	60 (7)
10^{-7}		102	93.1
10^{-6}		109	82.2
10^{-5}		132	124
10^{-4}		101	69.7

serum, in the presence or absence of cyclic AMP or the catecholamines. The values given are enzyme activities per milligram of total cell protein; since there is little change in cell number and amount of protein, they also represent the increase in total activity per petri dish. Similar results were obtained for epinephrine and cyclic AMP in 15 additional experiments (data not shown). Variation in duplicate assays was generally within 3 percent. The stimulation by isoproterenol and norepinephrine was even greater than that by epinephrine. The stimulation obtained with cyclic AMP and the catecholamines is similar to the effect obtained with HSF.

The β-receptor inhibitors, propanolol and dichloroisoproterenol (DCI), should prevent the epinephrine effect without interfering with the effect of cyclic AMP. Contrary to our expectations, we found that propanolol depressed the effects of both cyclic AMP and epinephrine. However, propanolol inhibited cellular myosin activity in the presence or absence of catecholamines, a result indicating a direct inhibitory effect of propanolol. It is not clear why propanolol was toxic, but it could not be used to differentiate the effects of epinephrine and cyclic AMP.

On the other hand, DCI acted as expected. In three experiments, DCI had no depressive effect on the stimulation caused by cyclic AMP except, in some cases, when DCI was used at four times the concentration of cyclic AMP. Two experiments are shown in Table 2. However, in four experiments, DCI reduced the epinephrine stimulation (two such experiments are shown in Table 2). In contrast to the propanolol toxicity, DCI alone, at four times the epinephrine concentration used in previous experiments, had no depressive effect on myosin activity (three experiments, not shown).

Epinephrine is generally described as an agent activating the organism in stress. It has profound effects on the heart, influencing the contractile force, synchronization, relaxation, and many metabolic activities (*7*). It is thought that most of the cardiac metabolic effects are mediated through the β receptors (*8*). In many tissues the β receptor stimulation is linked to the stimulation of adenylate cyclase (*9*). The same is true for the heart, where epinephrine increases the level of cyclic AMP (*10*).

There is a link between epinephrine and cyclic AMP with regard to metabolic effects such as increased phosphorylase (*8*), decreased glycogen synthetase (*11*), increased lipase activation (*12*), and increased uptake of free fatty acids (*13*) and oxygen (*14*). However, cyclic AMP may have many effects other than the well-known enzymatic activations. It is intimately related to the induction and transcription of β-galactosidase in *Escherichia coli* (*15*) and to the induction and translation of tryptophanase (*16*). Also, catecholamine and cyclic AMP have been implicated in the induction of lactic dehydrogenase in cultured glial cells (*17*) and in the synthesis of collagen in cartilage cells (*18*).

Epinephrine has a role in the stimulation of the heart activity, and cyclic AMP is also important in the regulation of myocardial contractility (*19*). Our data indicate their role in the maintenance of myosin. The HSF of serum stimulates the synthesis of myosin but does not protect against myosin degradation. We do not know whether the catecholamines and cyclic AMP act by increasing synthesis or by protecting against degradation, whether epinephrine directly stimulates myosin synthesis, or whether the mechanism of epinephrine activity is identical to that of HSF. In any case, however epinephrine achieves the increase in myosin activity, our results indicate that epinephrine activity, through its stimulation of adenylate cyclase, is important for the maintenance of myosin in the heart.

Table 2. Influence of dichloroisoproterenol (DCI) on effects of epinephrine and cyclic AMP. Experiments were done as in Table 1. In both experiments, determinations were made on the seventh day of incubation (the fourth day in the experimental medium). Concentrations of DCI were multiples of the concentrations of dibutyryl cyclic AMP (Dibu-cAMP) or epinephrine (Epi.); Exp., experiment.

Additions			Myosin adenosine triphosphatase (per milligram of total protein)	
DCI (M)	Dibu-cAMP (M)	Epi. (M)	Exp. 1	Exp. 2
0	0	0	130	31
0	10^{-4}	0	232	64
10^{-4}	10^{-4}	0	256	83
2×10^{-4}	10^{-4}	0	261	83
4×10^{-4}	10^{-4}	0	175	67
0	0	10^{-5}	179	48
10^{-5}	0	10^{-5}	152	62
2×10^{-5}	0	10^{-5}	106	27
4×10^{-5}	0	10^{-5}	80	18

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Thermophilic Ostracod: Aquatic Metazoan with the Highest Known Temperature Tolerance

Abstract. *The upper lethal temperature for an ostracod of the genus* Potamocypris *collected from a thermal stream ranged from 49°C for incubation of more than 5 hours to 55.75°C for 1-minute incubations. Field collections were held at 35°C for less than 24 hours before experimental incubations. Calculated temperatures for 50 percent mortality for 60, 40, 20, 10, 5, and 1 minute of exposure were 50.44°, 50.96°, 51.43°, 52.03°, 52.77°, and 55.12°C, respectively.*

Ostracods of the genus *Potamocypris* occur in some hot springs of western North America (*1, 2*) where they are found actively moving over the algal-bacterial substrate at temperatures of 30° to 54°C. Field observations on thermophilic animals (*2, 3*) consistently show the temperature limit for aquatic metazoans to lie below 50°C (*4*). This species of *Potamocypris* is found at higher temperatures than reported for other aquatic metazoans. Observations of live animals at a particular temperature are not proof of continued survival at that temperature (*5, 6*), so it was of interest to experimentally document the upper limits.

Mass collections of ostracods were made from water at 46°C in one of the thermal streams of the Hunter's Hot Springs complex located in south-central Oregon (*7*). Each collection was taken to the field laboratory and was kept at 35°C in a constant temperature bath until used (all ostracods were tested within 24 hours of collection). Adult ostracods were separated by pipette from the mass collection and subjected to the incubation conditions. Every trial consisted of placing ten adults into each of three test tubes containing 20 ml of spring water preheated to the desired temperature. The temperature was maintained in a Fisher serological bath and was continuously monitored with a thermistor probe placed in a test tube (*8*); no temperature fluctuation was observed during the reported trials of 1 hour or less (trials with temperature variations were terminated). After each incubation the test tubes were immediately cooled to 40° to 45°C by immersion in an ice slurry. Then the ostracods were removed and placed onto a depression plate and examined for active movement. Those not moving were placed in vials and kept at 35°C in the constant temperature bath for 12 hours, after which they were examined. Ostracods that were active after the recuperation time were considered to have been in heat coma. Those not regaining movement were considered dead.

In a preliminary experiment 60-minute incubations were made at 11 temperatures, from 40.00° to 50.00°C at 1° intervals. After this, with another group of animals, the incubations were for 1, 5, 10, 20, 40, and 60 minutes, from 48.00° to 55.75°C at 0.25° intervals. Including the preliminary incubations, replicates, and trials with 0 and 100 percent mortalities, a total of 2820 ostracods were tested. Replicate sublethal and lethal incubations during the trials served as controls. No thermal death or heat coma occurred below 49.50°C for these incubation periods.

Table 1 shows that as the exposure time is decreased the ostracod withstands proportionately higher temperatures. During 60-minute exposures both thermal coma and death were initiated at 49.50°C, but only at 51.00°C was mortality 100 percent in all trials. Heat coma was not observed until 53.75°C during 1-minute exposures, and no death occurred until the 54.50°C incubations.

A plot of trials falling within the thermal death ranges for the different exposure times is presented in Fig. 1, where each point represents a trial with 30 ostracods. Mortality slopes were determined by the least squares method (*9*). The regressions were significant at $P < .05$ [see Fig. 1 legend and (*10*)]. The values of the lethal dose to 50 percent of the population (LD_{50}) in Table 1 also show the expected relationship between temperature and incubation time. At $P < .05$ the LD_{50} value for 1 minute was significantly higher than those for all other exposure times except for 5 minutes; no other values were different at this level of significance (*10*), although they do show the expected trend.

The data scatter shown in Fig. 1, the relatively large overlapping thermal death ranges presented in Table 1, and the general absence of significant differences between the LD_{50} values indicate the highly variable response of the ostracods to most lethal temperatures. However, the low variability and statistical significance of the 1-minute exposures probably indicate that no

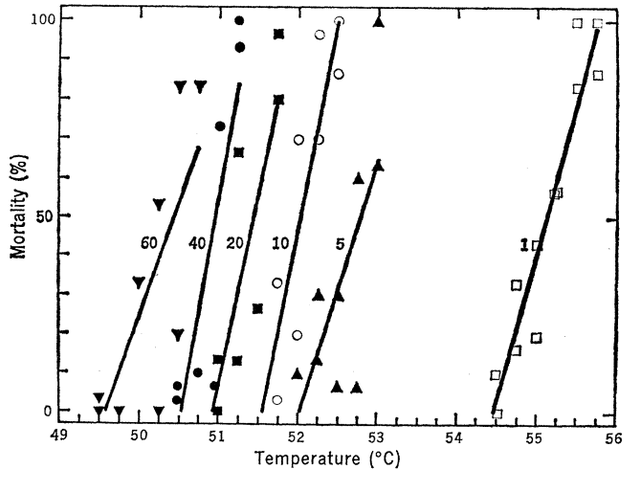

Fig. 1. Ostracod mortality within thermal death ranges. Each point represents 30 ostracods. (▼) 60-minute trials, $P < .02$; (●) 40 minute, $P < .02$; (■) 20 minute, $P < .05$; (○) 10 minute, $P < .01$; (▲) 5 minute, $P < .05$; (□) 1 minute, $P < .001$. Slope lines do not extend past the temperature above which no ostracods ever survived for that exposure period.