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Extracellular pH and Herpes Simplex Virus Multiplication.* (30365)

BERNARD ROIZMAN†

Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Md.

It is generally accepted that viruses furnish the infected cell with a template for duplication of their nucleic acid and with information for the synthesis of enzymes and of structural components of the virion. The synthesis of virus-directed nucleic acids and proteins takes place in the milieu of the infected cell and requires the participation of preformed enzymes, ribosomes, and other cellular constituents. Moreover, in both natural and experimental infections the cells are generally maintained under conditions suitable for growth or for survival. It could be expected, therefore, that similar environmental conditions would be required for optimal cell multiplication and for maximum yields of virus. However, this is not the case. Lwoff and Lwoff(1) have shown that the temperature of incubation for optimal poliovirus multiplication is genetically determined by the virus. The purpose of this communication is to report that in human epidermoid carcinoma No. 2 (HEp-2) cells the pH for optimal Herpes simplex virus multiplication is virus strain specific and in one instance substantially below the pH range necessary for growth and maintenance of host cells.

Materials and methods. Approximately 4-8 $\times$ 10^5 HEp-2 cells were exposed in phosphate buffered saline, pH 7.3, to sufficient virus to yield a multiplicity of infection of 10 to 30 plaque forming units per cell. After one hour of incubation at 37°C, the cells were washed twice with buffered saline containing antiviral antibody and once with saline alone. They were then incubated at 37°C in 8 ml of medium consisting of mixture 199 and 1% calf serum and buffered in pH range 5.5 to 8.0. The desired pH was obtained in two ways. In some experiments, media containing a constant amount of $NaHCO_3$ were flushed with 5 to 100% CO_2. In most experiments, media containing varying amounts of bicarbonate were flushed with air containing 10% CO_2. The pH was measured after equilibration of the media with CO_2, both at the beginning and at the end of each experiment. Three virus strains were used in these studies. Strain mP*dk*$^-$ was isolated from a patient with eczema herpeticum; in HEp-2 cell cultures it produces a microplaque consisting of a clump of rounded cells. MP*dk*$^-$ is a mutant derived from mP*dk*$^-$; it produces a macroplaque consisting of polykaryocyte(2). The mP*dk*$^-$ and MP*dk*$^-$ strains do not multiply in dog kidney (DK) cells. Strain MP*dk*$^+$*1p* is a multi-step mutant of MP*dk*$^-$;

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† Present address: Department of Microbiology, The University of Chicago, Chicago, Ill.

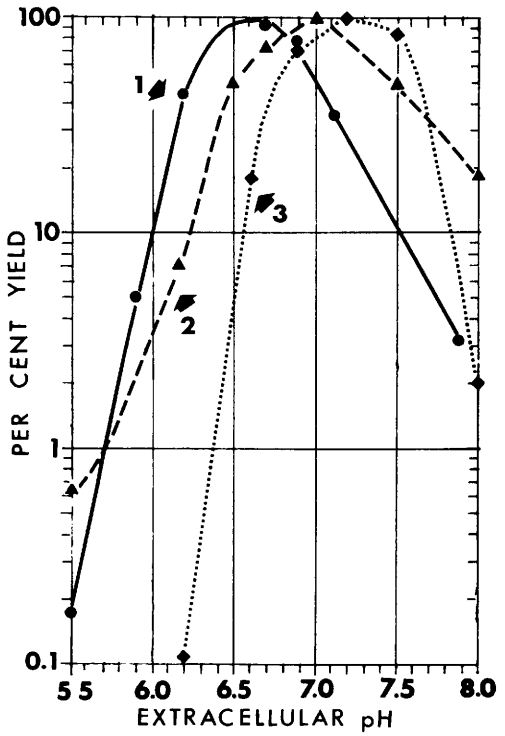

FIG. 1. Effect of extracellular pH on relative yields of 3 strains of Herpes simplex virus from HEp-2 cells 18 hr after infection. 1—Strain MP*dk*$^-$; 2—Strain mP*dk*$^-$; 3—Strain MP*dk*$^+$*1p*.

it multiplies and produces a large plaque in canine cells(3). The procedures for assays and pertinent properties of the 3 strains were described elsewhere(3,4). As a rule, 100 to 250 plaques were counted in each of replicate flasks. The standard error in these assays ranged from 4.4 to 7% of mean plaque count.

Results. The reproductive cycle of Herpes simplex virus strains used in these studies is 17 to 18 hours. Fig. 1 shows the relative yields of virus, both extracellular and intracellular, obtained in one experiment at the end of the reproductive cycle, *i.e.*, 18 hours after infection. The results were reproducible from experiment to experiment; they show that for each strain there is a distinct characteristic curve relating viral yield to extracellular pH. Thus, the extracellular pH for optimal multiplication of MP*dk*$^-$, mP*dk*$^-$, and of MP*dk*$^+$*1p* are 6.5-6.7, 7.0, and 7.2-7.3, respectively.

Discussion. The effects of extracellular pH on poliovirus multiplication have been studied by Dulbecco and Vogt(5). Their studies revealed that d$^+$ and d$^-$ mutants multiply equally well in cells incubated after infection in medium buffered at approximately pH 6.8. However, the d$^-$ multiply less well than d$^+$ in cells preincubated at that pH. The authors concluded that monkey kidney cells maintained at low pH are susceptible to d$^+$ but not to d$^-$ poliovirus mutants. The results reported here differ materially from those obtained with poliovirus mutants in that extracellular pH affects virus yields by modifying virus multiplication rather than cell infection. The processes of the reproductive cycle affected by pH are uncertain. It has been shown that mP*dk*$^-$, MP*dk*$^-$, and MP*dk*$^+$*1p* virions differ with respect to immunologic specificity, buoyant density in CsCl, stability on heating at 40°C, and effects on cells; these differences probably relate to the structure of the capsids(3,4). The differences among the 3 strains reported here may also relate to the structure of the capsids. Thus, it could be that the efficiency of polymerization of the subunits making up the capsids varies at different intracellular pH depending on the primary structure of the subunit.

The findings that (a) the extracellular pH for optimal virus multiplication is determined by the genetic constitution of the virus, (b) the extracellular pH for maximum yields of MP*dk*$^-$ virus from infected HEp-2 cells is 6.5-6.7, and (c) identical yields of MP*dk*$^-$ are obtained from infected cells incubated at pH 6.1 and 7.1 are significant from two points of view. First, it seems clear that HEp-2 and very likely other mammalian cells are capable of supporting viral macromolecular synthesis at a pH substantially below that necessary for optimal cellular macromolecular synthesis and multiplication. Second, the finding that the extracellular pH for optimal virus multiplication is determined by the genetic constitution of the virus has broad implication, particularly in the search for new viruses and in studies of viruses which multiply poorly under conditions which are optimal for their hosts.

Summary. Extracellular pH in the range 5.5 to 8.0 affects the yields of Herpes simplex virus obtained after one reproductive cycle in HEp-2 cells. For 3 strains of virus the extracellular pH for maximum yields are 6.5-6.7, 7.0, and 7.2-7.3, respectively. The data show that (a) extracellular pH for optimal virus multiplication is determined by the genetic constitution of the virus, and (b) HEp-2 cells support viral macromolecular synthesis when incubated in a medium buffered at pH as low as 6.0, *i.e.*, below the pH range for optimal cell multiplication and maintenance.

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Frog Gastric Mucosal ATPase.* (30366)

G. SACHS, W. E. MITCH† AND B. I. HIRSCHOWITZ
Department of Medicine, Division of Gastroenterology, University of Alabama Medical Center, Birmingham, Alabama

In recent years interest in extramitochondrial ATPases has centered in large part on 'transport' ATPases. These enzymes have been prepared from a variety of tissue—such as crab nerve(1), red blood cells(2), kidney (3), rat brain(4), and other tissues(5). They share a certain number of features in common, namely a stimulation by Na^+ and K^+ and at least partial inhibition by ouabain. Furthermore, in the standard cellular fractionation methods, the ouabain-inhibitable Na^+ and K^+ sensitive component occurs in the microsomal fraction which is considered to contain a large proportion of fragmented membrane. Further evidence for the involvement of the microsomal fraction in transport involves (a) ATP-dependent Na^+ binding to the protein of brain microsomes(4); (b) P^{32} labelling of protein derived from ATP^{32} in r.b.c. membranes(6) and (c) the reduction of labelled phosphoserine from kidney ATPase preparations incubated with DFP^{32} in the presence of ATP and a possible identity of this ATPase and diglyceride kinase(7).

The gastric mucosa under normal conditions apparently contains at least 2 electrogenic pumps acting on Cl^- and H^+ respectively(8). The specificity of the pumps in the gastric mucosa may make it extremely difficult to characterize a 'pump' ATPase in the same manner as has been done for those described above. Thus the chloride mechanism has been shown to transport several univalent anions in the mucosa and to have some action on sulfate(9). Therefore the anion pump may be regarded as relatively nonspecific. The hydrogen ion pump is apparently specific for H^+. A demonstration of H^+ activation is naturally complicated by the pH activity curves obtained for enzymic reactions. The evidence therefore for a hydrogen ion pump must at present rest on the action of specific inhibitors.

In this connection Kasbekar and Durbin (10) have recently described a SCN^- sensitive ATPase in the microsomal fraction of the gastric mucosa, and in view of the possible importance of this observation in relation to H^+ ion secretion mechanism, which is inhibited by SCN^-, some of the properties of the mucosal ATPases have been investigated with particular reference to the action of SCN^-.

Methods-materials. All chemicals were reagent grade. Nucleotides were purchased from Sigma Chemical Co., labelled nucleotides

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