Discussion. Although influenza viruses may be cultivated in a variety of primary cell cultures, including those derived from chick embryo lung and liver(7), and from the kidneys of many mammalian species(8), including man(9), they have not proved to be serially propagable in continuous cell lines, except as noted above. The present studies suggest that it is not the aneuploid nature of such cells that precludes their production of infective influenza virus because the diploid cell strains here employed were similarly deficient, yielding only incomplete virus with all strains other than NWS. The unique virulence and wide tissue tropism of NWS have been remarked before(8,1) and again are evident in the present findings.

It is possible that study of human diploid cells from organs other than the lung may reveal cell strains capable of supporting the multiplication of other influenza viruses. Studies with the rhinoviruses have demonstrated that the susceptibility of cells in primary cultures does not necessarily parallel the susceptibility of diploid cell strains from the same organ(10).

The differing susceptibility to NWS of the limited number of cell strains studied is of interest, and this finding supplements previous observations that diploid cell strains may differ in their susceptibility to certain rhinoviruses(11).

Summary. Inoculation with NWS of certain diploid cell strains derived from human embryonic lung resulted in production of infective and hemagglutinating virus coincident with induction of CPE. Diploid strains varied in susceptibility to infection with NWS virus. Influenza virus strains other than NWS did not induce the formation of infective virus and were not serially propagable in diploid cultures. However, in high concentration other influenza viruses induced CPE and the formation of incomplete virus.

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Disruption of Virus Biosynthesis *in vitro* with 6-mercaptopurine. I. Effect on DNA- and RNA- Viruses.* (29210)

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The success of 6-mercaptopurine in the treatment of some patients afflicted with plasma cell hepatitis, an illness possibly associated with persistence of virus in hepatic cells, has evoked speculation regarding an antiviral action of this purine analog(1).

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The simplicity of an *in vitro* system of host cell-virus interaction seemed the most appropriate method for investigation of this hypothesis.

Methods. Powdered 6-mercaptopurine (6-MP), Burroughs-Wellcome & Co., 15 to 30 mg, was solubilized in 1.0 ml 1 N NaOH and subsequently diluted in cell culture mainte-

TABLE I. Effect of 6-MP on Vaccinia Virus Plaque Formation in Strain Lass Cells.

Cell culture conc of 6-MP in μg/ml	Mean No. of plaques per replicate tube culture
30	0
15	0
5	0
3	0
1.5	0
.3	20
.03	38
None	37

nance medium (MM), consisting of 5% calf serum, Eagle's amino acid and vitamin mixture, Hanks' balanced salt solution (BSS), penicillin (100 units/ml), streptomycin (100 μg/ml) and sufficient sodium bicarbonate to provide a pH of 7.4-7.6. Appropriate dilutions of 6-MP were added to 0.9 ml volumes of MM nourishing tube cultures of host cells so that by 10-fold dilution the desired final culture concentration might be obtained.

The host cell in these experiments was the strain Lass cell(2), an established cell line derived from human liver, now continuously passaged in growth medium (GM) consisting of 20% calf serum, 0.1% yeast extract, Hanks' balanced salt solution, antibiotics, and sodium bicarbonate. Tube cultures of strain Lass cells were prepared so that by 48 hours confluent monolayers, composed of approximately 200,000 cells per 16 × 125 mm tube, were available.

Four virus seed pools were prepared: vaccinia virus (VV), avianized Lederle vaccine, propagated in strain Lass cells; herpes simplex virus (HSV), strain Brendes, obtained from corneal scrapings, propagated in strain Lass cells; measles virus (MV), strain Edmonston, obtained from Drs. J. M. Adams and D. T. Imagawa, propagated in strain HeLa cells; and poliovirus type 1 (T1), strain Mahoney, propagated in strain Lass cells.

In brief, virus assay was conducted in tube cultures of strain Lass cells by enumeration of macroscopic plaques (VV)(3), macro- and microscopic proliferative cytopathic plaque foci (HSV), microscopic syncytial cytopathic foci (MV), and by calculation of the 50% tissue (cell) culture infective (cytopathic) dose, $TCID_{50}$, using the method of Reed and Muench (T1)(4).

Results. Initial experiments involved the simultaneous addition of VV and 6-MP to strain Lass cells with subsequent enumeration of virus plaques after 48 hours of incubation at 37°C. Table I illustrates complete inhibition of VV plaque evolution by 6-MP at a final culture concentration of 1.5 μg/ml and greater, partial inhibition at 0.3 μg/ml, and absent inhibition at 0.03 μg/ml.

During the early phase of these studies the question arose concerning non-specific cell toxicity and lysis as being the crucial factor in inhibition of VV plaque formation. Accordingly replicate tube cultures of strain Lass cells were established in GM and 24 hours later several tubes removed, the monolayers trypsinized, and the suspended cells enumerated in a standard hemocytometer. With this tube culture cell population serving as the zero point, GM was removed, the cultures rinsed with BSS, then fed with fresh MM to which had been added appropriate dilutions of 6-MP so that final concentrations ranged from 0.3 to 50 μg/ml. In Fig. 1 it may be noted that a concentration of 6-MP which effects 50% VV plaque reduction fails to inhibit host cell multiplication over a 72-

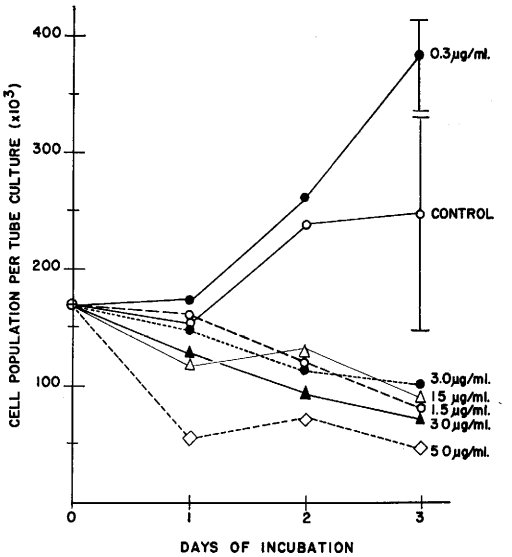

FIG. 1. Effect of 6-MP (final cell culture concentration) on multiplication of strain Lass cells.

hour period of cultivation at 37°C. Light microscopic observation of tube cultures revealed that monolayers exposed to 0.3 μg/ml of 6-MP were composed of cells identical in appearance to those of control cultures. The apparent inhibition of cell multiplication in tube cultures exposed to 1.5 and 3.0 μg/ml correlated well with minimal cellular degeneration and shedding as detected by light microscopy. Greater concentrations of 6-MP, 15 μg/ml and more, effected more significant alteration of both cell multiplication and microscopic morphology.

A final series of experiments was conducted to determine the spectrum of *in vitro* antiviral activity of 6-MP and to verify the fact that 6-MP indeed interfered with virus multiplication in host cells rather than simply with virus-induced cytopathic effect. By spectrum is inferred the alteration of *in vitro* infection with both DNA-viruses and RNA-viruses. VV and HSV comprised the DNA-viruses; MV and T1 the RNA-viruses(5). Tube cultures were inoculated with 40 plaque forming units (PFU) per 0.1 ml of VV and HSV and 100 $TCID_{50}$ per 0.1 ml of MV and T1. Immediately prior to virus inoculation control cultures were fed with MM, 1.0 ml, and experimental cultures with MM, 0.9 ml, to which was added 0.1 ml of 6-MP dilution containing 10 μg/ml, thus providing for these experiments a final cell culture concentration of 1.0 μg/ml 6-MP. After 72 hours of incubation at 37°C either supernatant fluid (HSV, T1) or cells plus fluid (VV, MV) were harvested from both control and experimental cultures. These culture harvests were subjected to 3 cycles of freezing and thawing, and 0.1 ml volumes of subsequent, appropriate dilutions assayed per methods above. Tables II-IV indicate that 6-MP evokes striking inhibition of replication of VV (800-fold) and HSV (300-fold) and significant inhibition of MV (17-fold) and T1 (8-fold) in strain Lass cells.

TABLE II. Effect of 6-MP on Replication of Vaccinia Virus in Strain Lass Cells—72 Hr.

Inoculum, 40 PFU/.1 ml	Mean No. of plaques per .1 ml harvest—cells plus supernatant fluid
Control cultures, without 6-MP	4000
Experimental cultures, 6-MP, 1 μg/ml	5

TABLE III. Effect of 6-MP on Replication of Herpes Simplex Virus in Strain Lass Cells—72 Hr.

Inoculum, 40 PFU/.1 ml	Mean No. of plaques per .1 ml harvest—supernatant fluid
Control cultures, without 6-MP	300
Experimental cultures, 6-MP, 1 μg/ml	0

TABLE IV. Effect of 6-MP on Replication of Measles Virus in Strain Lass Cells—72 Hr.

Inoculum, 100 $TCID_{50}$/.1 ml	Mean No. of syncytial foci per .1 ml harvest—cells plus supernatant fluid
Control cultures, without 6-MP	17
Experimental cultures, 6-MP, 1 μg/ml	0

TABLE V. Effect of 6-MP on Replication of Poliovirus, Type 1, in Strain Lass Cells—72 Hr.

Inoculum, 100 $TCID_{50}$/.1 ml	Log titer in $TCID_{50}$ per .1 ml harvest—supernatant fluid
Control cultures, without 6-MP	$10^{3.9}$
Experimental cultures, 6-MP, 1 μg/ml	$10^{3.0}$

Discussion. That 6-MP disrupts the virus-host cell relationship at a concentration which spares the integrity of the host cell appears indisputable from experiments with VV. Experiments utilizing concentrations of 6-MP which alter host cell population and morphology to a moderate degree indicate that both DNA-viruses and RNA-viruses are susceptible to this disruptive effect. Although conceivably a function of replication kinetics (MV) or assay techniques (T1), the RNA-viruses studied seem less susceptible to *in vitro* inhibition. Such a spectrum of inhibition is encouraging because the pyrimidine analogues, 5-bromo-, 5-fluoro-, and 5-iododeoxyuridine, and the antimetabolites, aminopterin and actinomycin D, impair the multiplication of only DNA-viruses(6-9). Only the benzimidazole compound, 5,6-dichloro-1-β-d-ribofuranosylbenzimidazole, studied by

Tamm and his associates, interferes with the replication of both DNA- and RNA-viruses (10).

The fact that its biologic effect can be so easily assayed *in vitro* via inhibition of VV plaque formation supplies an additional laboratory tool for elucidation of the biochemical mechanism of action of 6-MP.

Additional important parameters remain to be fully evaluated, *i.e.*, the phase during virus-host cell interaction at which 6-MP operates, the ability of 6-MP to interrupt *in vitro* virus infection once well established, and finally and most crucially the response of virus infection *in vivo* to 6-MP therapy. These areas are being investigated.

Summary. *In vitro* experiments with 6-mercaptopurine have demonstrated that concentrations of the purine analog as low as 1 μg/ml effectively disrupt the multiplication of both DNA-viruses (vaccinia, herpes simplex) and RNA-viruses (measles, polio) in established strain Lass cells.

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Sudden Unexpected Death in Infancy. Isolations of ECHO Type 7 Virus. (29211)

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Sudden and unexpected death in infancy (crib death) has become a public health problem of considerable magnitude. The almost classic symptomatology has been adequately described by several investigators(1, 2), where an apparently well baby with or without mild respiratory difficulties is found dead in its crib. Viral etiology was suggested as early as 20 years ago(3); recent reviews (4,5), however, have shown a paucity of isolations from autopsy material. The following paper presents studies on isolation of viruses from 7 of 10 crib deaths, obtained randomly within a 12-month period from the metropolitan Phoenix area.

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Materials and methods. Tissue cultures. Preparation and history of human embryonic epithelial lung cultures (HEL) have been reported elsewhere(6). For use in these studies, approximately 200,000 cells per ml suspended in 20% calf serum-M199 were dispensed into screw-cap roller tubes. Confluent sheets of cells were observed within 18 to 24 hours, at which time the medium was replaced with 2% calf serum-M199 for maintenance. Primary renal cultures obtained from the grivet (*Cercopithecus aethiops*) were prepared according to methods described for preparation of rhesus monkey kidney cell cultures(7). Kidney cell roller cultures were usually ready to use in 5 to 7 days, at which time fresh 2% calf serum-M199 was substituted for maintenance medium.

Prototype enterovirus serums were ob-