tude of the differences observed. Variation of enzyme level with age is a possible explanation. The birds used in this study were 10 days old and therefore considerably younger than those used in other studies. This is apparently the case with alkaline phosphatase level, which is markedly elevated at 1 and 2 weeks as compared to 6 weeks of age(17,18). Age differences, as opposed to species differences, might also account for the higher levels of leucine aminopeptidase and amylase obtained in chickens than humans.

Summary. Level of 10 enzymes was measured in sera of 10-day-old females from lines resistant and susceptible to leucosis. The level of cathepsin was significantly higher in the resistant line ($P<.01$); similar results were also obtained at 4 weeks of age. No significant and repeatable differences were observed in the other enzymes.

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Serial Multiplication of an Influenza Virus (NWS) in Certain Human Diploid Cell Strains.* (29209)

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Evidence for the serial multiplication of influenza viruses in continuous aneuploid cell lines has been published in only two reports (1,2). Brief mention has been made of the cytopathic effect of 3 influenza viruses in the WI-1 human diploid cell strain, but evidence of viral multiplication or other experimental details was not provided(3). Evidence is presented here that the neurotropic WS strain (NWS) of influenza A virus can multiply with the production of infective virus in certain diploid cell strains derived from human embryonic lung. The formation of hemagglutinating but noninfective virus following inoculation of other strains of influenza virus will also be described.

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Materials and methods. Viruses. Variants of the Stuart-Harris neurotropic strain of the WS influenza A virus (NWS) and of the S-15 strain of swine influenza virus which had re-

TABLE I. Diploid Cell Strains Used.

Cell strain	Passage No. used	Tissue origin	Source
Wyeth 3	6,13,14	HEL*	Wyeth Labs
" 1024	15,19,22	"	" "
" 1129	11	"	" "
M-7	17	"	Microbiol. Assoc.
WI-26	28	"	" "
WI-38	20	"	Wyeth Labs

* HEL—human embryo lung.

ceived passage in a variant of the Chang conjunctival cell were used in several experiments. These viral variants have been designated NWS-cc and Swine-cc, respectively(1). In addition, the standard egg-passaged strain of NWS was also employed. Other influenza virus strains that were used included the PR8 strain of influenza A, the RI/5+ strain of influenza A2(4) and the Lee strain of influenza B virus.

Cell strains. Data concerning the cell strains used are summarized in Table I. All strains exhibited the histotypical differentiation characteristic of diploid strains(3), were in relatively early passage, and showed no evidence of the degenerative changes in morphology which accompany senescence of diploid cultures. "Diploid" cells of less than 40 passages have been found to retain the normal chromosome complement(5). Cells of the Wyeth strains and the WI-38 strain used in the present experiments were grown in Hanks'-BME with glucose 1 mg/ml, $NaHCO_3$ 0.075%, and phenol red 0.001%, and calf serum 10% in 15 ml test tubes with gas permeable stainless steel closures; tubes were incubated at 37°C in an atmosphere of CO_2 estimated as 5%. Tubes were seeded with 10^5 cells/tube. After a 4-5 day growth period, intact monolayers were found, in one experiment, to contain 10^5 cells. The M-7 and WI-26 strains were received as tube cultures from Microbiological Associates, Inc. All cell strains were maintained in 199 with final $NaHCO_3$ concentration of 0.0188-0.0375% and with 2% normal horse serum previously heated at 56°C for 30 minutes.

Virus titrations. Virus quantitation was carried out by methods described elsewhere (1).

Results. Evidence for serial passage and multiplication of NWS virus. When 10^5 cells of the Wyeth-3 strain were inoculated with $10^{6.3}$ EID_{50} of NWS-cc virus (an estimated input multiplicity of 20 EID_{50}/cell) cytonecrosis appeared between the fourth and seventh day. Culture fluids agglutinated human O erythrocytes and were found to contain $10^{5.5}$ EID_{50} of virus per ml (Table II). Two subsequent serial passages of virus in this cell strain reached a cumulative dilution calculated to leave only 2 of the original egg infective particles, despite a cumulative incubation time of 21 days. Complete thermal inactivation of infectivity of this heat labile strain is predictable on the basis of earlier studies in this laboratory. More conclusive, however, is the evidence for multiplication provided by sub-inoculation of eggs; fluids from the second passage of virus in the Wyeth-3 cells contained more infective virus than had been originally inoculated. The further passage of virus in another diploid strain (Wyeth-1024) with evidence of production of infective virus in these cells is also shown in Table II. In this Table, the log of the viral inoculum in cell culture represents the least amount of virus necessary to induce detectable hemagglutinin formation. It is thus apparent that under the experimental conditions defined, the cultures were 100-1000 times less susceptible to the virus which they produced than was the chick embryo.

In other experiments with the Wyeth-1024 strain, it has been shown that prior passage of the NWS strain in human conjunctival cells is not a requisite for serial propagation of the virus in these cells.

The differing capacity of influenza virus

TABLE II. Evidence for Serial Passage and Multiplication of NWS-cc Virus in Human Diploid Cell Strains.

	Cell strain	Log N viral inoculum*	Log N virus yield/ml HA	Log N virus yield/ml EID_{50}
Passage 1	Wyeth 3	6.3	1.3	5.5
2	"	2.5	2.1	7.0
3	"	2.0	.6†	—
3	Wyeth 1024	3.0	.6†	7.0
4	"	3.0	.6*	—

* EID_{50}.
† Maximal dilution tested for HA was 1:4.

TABLE III. Differing Capacity of Various Influenza Virus Strains to Induce CPE and HA Formation in a Strain of Human Diploid Cells (Wyeth 3).

Virus	Minimal virus dose* needed to induce: CPE	HA	MHA	EID_{50} of cultures positive for HA at minimal virus dose: Yield
NWS-cc	3.5	3.5	N.D.	7.0/ml*
NWS-cc-dip-1	>2.5	2.5	2.5	5.5 "
NWS-cc-dip-2	4.0	2.0	2.0	7.0 "
PR 8	7.5	7.5	7.5	—
RI/5†	>4.7	>4.7	>4.7	—
Lee	5.7	5.7	5.7	<2.0/0.1 ml
Swine-cc	>6.0	6.0†	6.0†	<2.0 " "

* Log N EID_{50}.
† Only dose employed.
CPE = cytopathic effect.
HA = hemagglutinin at 1:4 dilution or greater of culture fluid.
MHA = hemadsorption.
NWS-cc-dip-1 = virus derived from 1 passage in diploid cell culture.

strains to induce cytopathic effects and hemagglutinin formation in a human diploid cell strain. In Table III are presented the results of inoculation of cultures of one cell strain—Wyeth-3—with titrated doses of several influenza viruses. Results are expressed as the minimal dose of virus required to induce these effects. With the 3 sub-strains of NWS employed, 10^2 to $10^{3.5}$ EID_{50} were required to induce hemagglutinin formation, while doses of $10^{4.8}$ of more were needed of the other strains tested. Furthermore, passage of HA positive fluids from tubes inoculated with PR8, Lee, RI/5+ or the Swine-cc strains failed to induce CPE or HA in Wyeth-3 cell cultures. In the case of Lee and Swine-cc viruses it was also demonstrated that HA positive fluids contained less than 10^2 EID_{50} per 0.1 ml. In contrast, inoculation of chick embryos with hemagglutinin which appeared following inoculation of cultures with NWS sub-strains disclosed the presence of $10^{5.5}$-$10^{7.0}$ EID_{50} of virus per ml. It was concluded that the strains other than NWS had induced the formation of hemagglutinating but non-infective (incomplete) virus as previously observed in cultures of aneuploid cell lines(6).

Differing susceptibility to NWS-cc virus of several diploid cell strains derived from human embryo lung. A difference in the susceptibility among several diploid cell strains to the effects of the same virus, NWS-cc, was noted. This difference was reflected by the amount of virus required for induction of CPE and formation of hemagglutinin, and in the yield of egg infective virus. By these criteria the Wyeth cell strains ranged from a state of susceptibility comparable to the chick embryo (Wyeth-1024) through intermediate susceptibility (Wyeth-3) to the relatively refractory state (Wyeth-1129) which also characterized the M-7, WI-26 and WI-38 strains (Table IV). With the Wyeth-1129 and WI-26 strains evidence of infective virus production was equivocal since an input dose of $10^{5.3}$ resulted in a yield of only $10^{4.7}$ EID_{50}. However, thermal inactivation of virus during the 7-day experimental period undoubtedly exceeded the 0.6 log drop in titer, so that multiplication probably occurred.

TABLE IV. Differing Susceptibility to NWS-cc Virus of Human Embryonic Lung Diploid Cell Strains.

Cell strain	Passage No.	Minimal virus dose* needed to induce: CPE	HA	MHA	EID_{50} of cultures positive for HA at minimal virus dose*: Yield/ml
Wyeth 3	13	3.5	3.5	N.D.	7.0
" 1024	19	.4	.4	.4	—
" 1129	11	6.3	5.3	N.D.	4.7
M-7	17	6.3	5.3	5.3	—
WI-26	28	5.3	5.3	5.3	4.7
WI-38	20	5.0	6.0	3.0	—

* Virus NWS-cc; dose = log N EID_{50}.
CPE = cytopathic effect.
HA = hemagglutinin at 1:4 dilution or greater of culture fluid.
MHA = hemadsorption.

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-