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Effect of 5-Fluoro-2′-Deoxyuridine on the Synthesis of Vaccinia Virus[1]

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The effect of FUDR on vaccinia virus replication in HeLa cells was investigated. The incorporation of deoxyuridine-H^3 into viral DNA, as determined by autoradiography, was completely blocked by 10^{-6} *M* FUDR. This concentration of the analog prevented production of infectious virus above levels present in the cultures after washing at the end of the viral adsorption period. The production of vaccinial HA was also inhibited. However, 20–70% of the inhibited cells produced NP and LS antigen detectable by the fluorescent antibody technique. These findings were in accord with the results of previous experiments using *p*-fluorophenylalanine which had suggested that viral antigen can be synthesized in the absence of synthesis of viral DNA.

The proportion of cells that synthesized antigen was progressively reduced by increasing the concentration of FUDR or increasing the duration of pretreatment of cultures with the analog. The inhibition of synthesis of infectious virus and of viral antigen was not prevented by uridine added with the FUDR but was reversed by thymidylic acid added at the time of infection to cultures that had been pretreated for as long as 12 hours with FUDR. When FUDR was added to cultures at the time of infection and thymidylic acid was added 12 hours later, the proportion of cells that synthesized viral antigen was essentially the same as in cultures not treated with FUDR; however, the yield of infectious virus in the former cultures was only 15% of that from cultures not treated with the analog.

INTRODUCTION

It was observed (Loh and Payne, 1965) that the amino acid analog *p*-fluorophenylalanine inhibited the synthesis of vaccinal DNA,[3] NP antigen (Smadel *et al.*, 1942), infectious virus, and vaccinial HA. However, under conditions where *p*-fluorophenylalanine inhibited viral DNA synthesis, many infected cells produced what appeared to be large amounts of LS antigen (Shedlovsky and Smadel, 1942). The present study was undertaken to examine further the production of viral materials by vaccinia-infected cells in which viral DNA synthesis was inhibited.

It had been reported that treatment of bacterial or mammalian cells with fluorinated pyrimidines inhibited DNA synthesis while RNA and protein synthesis continued (Cohen *et al.*, 1958; Bosch *et al.*, 1958; Harbers *et al.*, 1959; Salzman, 1960). 5-Fluoro-2′-deoxyuridine (FUDR), which blocks the formation of thymidylic acid and

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[3] Abbreviations: BME, basal medium Eagle's; BSS, Hanks' balanced salt solution; DNA, deoxyribonucleic acid; EM, experimental medium; FUDR, 5-fluoro-2′-deoxyuridine; HA, hemagglutinin; HAU, hemagglutination unit; LS antigen, heat-labile-heat-stable antigen; NP antigen, nucleoprotein antigen; PFU, pock-forming unit; p.i., post-infection; RNA, ribonucleic acid; TMP, thymidylic acid.

consequently inhibits the synthesis of DNA (Cohen *et al.*, 1958; Bosch *et al.*, 1958) had been found to inhibit the replication of several viruses containing DNA (Salzman, 1960; Flanagan and Ginsberg, 1961; Simon, 1961). The objective of the experiments to be reported was to inhibit viral DNA synthesis with FUDR without markedly interfering with the synthesis of RNA or protein and thus determine whether DNA synthesis was required for the production of viral antigens.

Subsequent to the completion of this study Shatkin (1963) and Easterbrook (1963) reported that some vaccinia virus protein is formed in the presence of FUDR.

MATERIALS AND METHODS

Unless otherwise noted all materials and methods used in the present investigation were those described in the preceding paper (Loh and Payne, 1965). The limitations of immunofluorescent staining as a quantitative technique were discussed in the preceding paper and should be kept in mind in considering the data to be presented. It should be emphasized that the presence of the vaccinial nucleoprotein, NP antigen, as determined by staining with specific fluorescein-conjugated gamma globulins, does not imply the presence of viral DNA.

Experimental procedure. Stationary tube cultures, containing approximately 10^5 HeLa cells, were washed with three 1-ml portions of BSS and changed to 1 ml of the experimental medium (EM) consisting of BME and 2% equine serum. Cultures were infected by incubation for 2 hours at 37°C with 1 ml of EM containing virus at a multiplicity of exposure of approximately 20 PFU per cell. At the end of this period unadsorbed virus was removed by washing the cultures with three 1-ml portions of BSS. One milliliter of EM was then added, and the cultures were incubated further at 37°. The time at which virus was added to the cultures was considered 0 time.

At times indicated in the individual experiments various concentrations of FUDR were added to the EM. When cultures were treated with FUDR during the viral adsorption period, the BSS used to wash the cultures at the end of this period and the EM subsequently added contained the indicated concentration of the analog. When TMP, uridine, or deoxyuridine-H^3 were used during the adsorption period they were also present in the medium during subsequent incubation of the culture.

Control cultures were handled in the same manner as those described above except that virus, chemical, or both, as indicated, were not added to the medium.

For determination of the proportion of cells containing antigen cultures were washed with BSS and fixed in acetone at 10 hours p.i. unless otherwise noted. Yield of infectious virus and HA were determined on samples from cultures which were frozen at 24 hours p.i.

Chemicals. The pyrimidine analog FUDR, was generously supplied by the Cancer Chemotherapy Service Center, National Institutes of Health, Bethesda, Maryland. Thymidylic acid and uridine were obtained from the California Corportation for Biochemical Research. For each experiment freshly prepared solutions of each compound were used.

Tritiated deoxyuridine (specific activity = 1.5 curies/mmole) was used at a concentration of 5.0 μc per milliliter of culture medium. This labeled compound was obtained from New England Nuclear Corporation.

RESULTS

Effect of FUDR on the Formation of Vaccinial Antigens and Infectious Virus

FUDR in varying concentrations was added with vaccinia virus to a series of replicate HeLa cell cultures. At 10 hours post-infection (p.i.) coverslip preparations were fixed for staining with fluorescent antibody and enumeration of cells containing viral antigen. Yields of infectious virus and HA were determined on samples from cultures incubated until 24 hours p.i.

As shown in Fig. 1, cultures treated with 10^{-7} M FUDR yielded only 2.5% as much infectious virus as untreated infected cultures. With concentrations of 10^{-6} M or higher, the amount of virus per culture at 24 hours p.i. was similar to that present

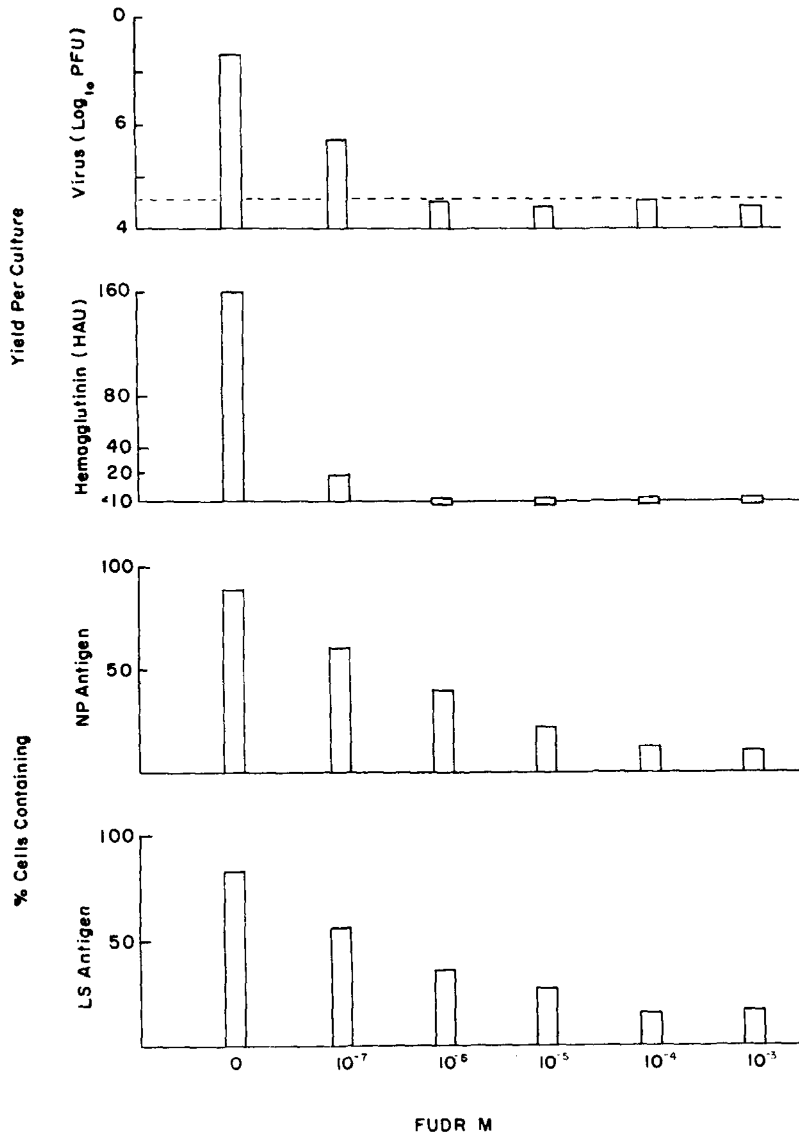

FIG. 1. The effect of FUDR on the formation of vaccinia virus, vaccinial hemagglutinin, NP antigen, and LS antigen in HeLa cells. Replicate cultures were infected as described in Materials and Methods. FUDR, to give the indicated concentration was added to cultures at the same time as the virus. The proportion of cells containing viral antigen was determined using coverslip preparations fixed at 10 hours p.i. The yields of infectious virus and HA were determined on cultures frozen at 24 hours p.i. *(------) represents the virus per culture present after washing at the end of the 2-hour adsorption period.

after washing the cultures at the end of the 2-hour adsorption period and approximately 0.1% of that present at 24 hours p.i. in cultures not inhibited by FUDR.

The production of HA was also inhibited by FUDR. Approximately 10% of the expected yield of HA was found in cultures treated with 10^{-7} *M* FUDR and, within the limit of the test (approximately 5% of the yield from cultures not treated with FUDR), no HA was detected at 24 hours p.i. in cultures treated with the analog at concentrations of 10^{-6} *M* or greater.

Whereas FUDR at concentrations of 10^{-6} *M* or greater inhibited all detectable production of virus and HA, it inhibited only par-

TABLE 1

EFFECT OF 5-FLUORO-2′-DEOXYURIDINE ON INCORPORATION OF DEOXYURIDINE-H^3 INTO NUCLEAR AND CYTOPLASMIC DNA OF UNINFECTED AND VACCINIA VIRUS-INFECTED HELA CELLS AS DETERMINED BY AUTORADIOGRAPHY[a]

Culture	Interval of exposure to deoxyuridine-H^3 (hours p.i.)	% Cells with label	
		Nuclear	Cytoplasmic
Uninfected	0–5.0	44	0
Uninfected + FUDR	0–5.0	0	0
Infected	0–5.0	22	72
Infected + FUDR	0–5.0	0	0
Uninfected	4.0–8.0	35	0
Uninfected + FUDR	4.0–8.0	0	0
Infected	4.0–8.0	8.5	63
Infected + FUDR	4.0–8.0	0	0

[a] Cultures were infected as described in Materials and Methods. FUDR to give a concentration of 10^{-6} M was added to the indicated cultures at the same time as the virus. Cultures were exposed to deoxyuridine-H^3 (5.0 μc/ml) for the indicated interval. At the end of this interval cells on coverslips were fixed for autoradiography and determination of the proportion of cells containing acid-insoluble H^3 in their nuclei or cytoplasm.

tially the formation of LS and NP antigen. The proportion of cells containing NP or LS antigen decreased progressively with concentrations of FUDR from 10^{-7} M to 10^{-3} M. Cells that produced these antigens in the presence of FUDR appeared to contain less antigen than cells not treated with the analog. The inclusions in FUDR-treated cells were generally smaller and stained less intensely with fluorescein-labeled antibody than those in untreated infected cells. This observation was in contrast to the previous finding (Loh and Payne, 1965) that cells which were able to produce NP or LS antigen in the presence of *p*-fluorophenylalanine contained what appeared to be as much antigen as the untreated controls.

It should be noted that when FUDR (10^{-6} M) was added at 0 time, the cultures, which contained approximately 10^5 cells, produced less than 0.1 PFU per cell. The proportion of cells yielding infectious virus under these conditions could not have exceeded 10% if each PFU had been produced by a different cell. Since in these cultures 40% of the cells had produced LS and NP antigen, it appeared that cells that produced no detectable infectious virus had produced viral antigen.

In other experiments with FUDR, added at 0 time to give a concentration of 10^{-6} M, the proportion of cells containing antigen at 10 hours p.i. varied from 20 to 70%; however, the yield of infectious virus from such cultures was consistently no more than that amount present after washing the cultures at the end of the 2-hour viral adsorption period.

Effect of FUDR on the Utilization of Deoxyuridine in the Synthesis of DNA

Because 10^{-6} M was the lowest concentration of FUDR that markedly inhibited production of infectious vaccinia virus and yet allowed many cells to produce viral antigen, the effectiveness of this concentration of the analog in blocking the conversion of deoxyuridylic acid to thymidylic acid was examined.

HeLa cells, noninfected and infected with vaccinia virus, were incubated with and without FUDR from time zero. The incorporation of deoxyuridine-H^3 into acid-insoluble material of the nucleus (cellular DNA) and of the cytoplasm (viral DNA) was determined by autoradiography. It had been found previously that the majority of the infected cells were synthesizing viral DNA by 4.0 to 4.5 hours p.i. (Loh and Payne, 1965). In the present experiment, cultures were exposed to deoxyuridine-H^3 from 0 to 5.0 hours p.i. or from 4.0 to 8.0 hours p.i.

The results presented in Table 1 indicate that in HeLa cells deoxyuridine-H^3 was utilized as a precursor for the synthesis of cellular (nuclear) and viral (cytoplasmic) DNA. In uninfected cultures the label was limited to the nucleus, but infected cultures contained label in cytoplasmic inclusions and the nucleus. With uninfected and infected cells treated with FUDR and exposed to deoxyuridine-H^3 for either 0–5.0 hours p.i. or 4.0–8.0 hours p.i. no label was seen in either the nucleus or cytoplasm. Thus,

FUDR at 10^{-6} *M* concentration had produced an immediate and complete block in the conversion of deoxyuridine to thymidylic acid and hence the incorporation of deoxyuridine into cellular or viral DNA.

If there had been a pool of preformed thymidine derivatives in the HeLa cells, FUDR would not be expected to have interfered with its use for DNA synthesis. For this reason, the possibility that some viral DNA synthesis had occurred after the addition of FUDR to the cultures was further examined.

Effect of Pretreatment of HeLa Cells with FUDR on the Synthesis of Infectious Virus and NP Antigen

One might expect that infectious virus produced by FUDR-inhibited cultures would represent not only the amount of viral DNA synthesized prior to the addition of the analog, as suggested by Salzman (1960), but also viral DNA synthesized from thymidine derivatives that might be present at the time the analog was added. Incubation of cultures with FUDR for various periods prior to infection would be expected to allow depletion of these precursors of DNA and consequently lower the virus yields. To examine this possibility cultures were incubated with FUDR (10^{-6} *M*) for various periods prior to addition of virus and maintained in this concentration of inhibitor throughout the experiment. The cultures were washed after a 2-hour viral adsorption period and returned to EM containing inhibitor for further incubation at 37°. Virus yields were determined on samples taken at 24 hours p.i.

As seen in Table 2, cultures treated with FUDR as long as 12 hours prior to infection contained at 24 hours p.i. the same amount of infectious virus as did cultures treated at 0 time. The same results were obtained with pretreatment with 10^{-4} *M* FUDR. Thus, by this technique no evidence was found to indicate the existence of a pool of preformed thymidine derivatives. If, however, the virus present at 24 hours p.i. in cultures receiving FUDR at 0 to −12 hours p.i. represented residual viral inoculum, this would have masked smaller amounts of virus, of the order of 1000 PFU per culture, that might have been synthesized from preformed thymidine derivatives.

TABLE 2

EFFECT OF PREINCUBATION OF HELA CELLS WITH 5 - FLUORO - 2′ - DEOXYURIDINE, WITH AND WITHOUT ADDED URIDINE, ON THE PRODUCTION OF VACCINIA VIRUS AND NP ANTIGEN[a]

Expt. no.	Compound added	Time compound added (hours before infection)	Virus yield per culture ($\log_{10}$ PFU)	% Cells containing NP antigen
I	FUDR	0	4.33	20
I	FUDR	3	—	16
I	FUDR	6	4.40	10
I	FUDR	12	4.37	2
I	None	—	7.35	93
II	FUDR	0	4.45	28
II	FUDR + U	0	4.30	30
II	None	—	7.30	82

[a] FUDR, to give a concentration of 10^{-6} *M*, was added to cultures at the indicated times. Uridine (U) was added to give a concentration of 10^{-4} *M*. Cultures were infected as described in Materials and Methods. In those cultures receiving FUDR or U, these compounds were present during the viral adsorption period and the subsequent period of incubation. The proportion of cells containing antigen and the yield of infectious virus were determined on the cultures at 10 and 24 hours p.i., respectively.

It was found (Table 2) that cultures from the above experiment fixed at 10 hours p.i. yielded progressively fewer cells containing NP antigen as the period of pretreatment with FUDR was increased. In the absence of evidence indicating that preformed thymidine derivatives were present in the cultures which might have been responsible for limited synthesis of viral DNA and antigens, it was necessary to consider mechanisms other than inhibition of viral DNA synthesis by which FUDR could interfere with antigen synthesis.

Effect of Uridine on FUDR-Inhibition of Synthesis of Vaccinia Virus

FUDR might have been converted to fluorouracil or its riboside derivatives,

thereby affecting RNA and possibly protein synthesis. To examine this possibility, the effect of FUDR on the production of infectious vaccinia virus and NP antigen in HeLa cells in the presence and absence of uridine was investigated. Uridine would be converted to uridylic acid which would compete with fluorouridylic acid in the synthesis of RNA. If FUDR interferes with the synthesis of virus or NP antigen by such a secondary effect on RNA synthesis, uridine would be expected to prevent this effect.

Vaccinia virus-infected cultures were incubated from 0 time with 10^{-6} *M* FUDR in the presence and absence of 10^{-4} *M* uridine. The proportion of cells containing NP antigen was determined on samples taken at 10 hours p.i. and the virus yield at 24 hours p.i. The amount of infectious virus or the proportion of cells containing NP antigen in FUDR-treated cultures was not increased in the presence of uridine (see Table 2).

Effect of Thymidylic Acid on FUDR-Inhibition of Synthesis of Vaccinia Virus

FUDR had been reported to block the synthesis of thymidylic acid, but not to interfere with utilization of TMP for the synthesis of DNA (Cohen *et al.*, 1958; Bosch *et al.*, 1958). Hence, to examine the possible role of nonspecific damage to cells as a factor in inhibition of synthesis of viral antigen, the ability of TMP to reverse the inhibition of viral synthesis was examined.

FUDR (10^{-6} *M*) was added to one set of cultures at the time of infection and to another at 12 hours before infection. TMP (6.4×10^{-4} *M*) was added to both sets at the time of infection. Other cultures received FUDR at the time of infection, and TMP was added at 12 hours p.i. The proportion of cells containing NP antigen was determined on samples taken at 10 hours p.i. or 10 hours after the addition of TMP. Samples were frozen at 24 hours p.i. or 24 hours after TMP was added for subsequent measurement of infectious virus and HA. The results are presented in Table 3.

In terms of the proportion of cells synthesizing NP antigen, the inhibition of synthesis of antigen was reversed by TMP added to cultures treated with FUDR for as long as 12 hours either before or after infection. Although the inhibition of synthesis of infectious virus and HA was com-

TABLE 3

EFFECT OF THYMIDYLIC ACID ON THE FUDR-INHIBITION OF SYNTHESIS OF VACCINIA VIRUS, NP ANTIGEN, AND VACCINIAL HEMAGGLUTININ IN HELA CELLS[a]

Expt. No.	Compound added	Virus yield per culture ($\log_{10}$ PFU)	% Cells containing NP antigen	Hemagglutinin yield per culture (HAU)
I	None	7.30	82	160
I	FUDR at 0 time	4.18	28	<10
I	FUDR and TMP at 0 time	7.35	80	160
I	FUDR at −12 hr p.i.	4.20	8	<10
I	FUDR at −12 hr p.i. and TMP at 0 time	7.28	83	160
II	None	7.64	84	160
II	FUDR at 0 time	4.62	34	<10
II	FUDR and TMP at 0 time	7.67	81	160
II	FUDR at 0 time and TMP at +12 hr p.i.	6.83	78	80

[a] FUDR, to give a concentration of 10^{-6} *M*, was added to cultures at the time of infection (0 time) or 12 hours before infection (−12 hr p.i.). Thymidylic acid (TMP) was added at 0 time or at 12 hours after infection (+12 hr p.i.) to give a concentration of 6.4×10^{-4} *M*. Cultures were infected as described in Materials and Methods. When FUDR or TMP were present during the viral adsorption period, they were also present during the subsequent period of incubation. The proportion of cells containing NP antigen was determined using coverslip preparations fixed at 10 hours p.i. or 10 hours after the addition of TMP. Cultures were frozen at 24 hours p.i. or 24 hours after the addition of TMP for measurement of the yields of infectious virus and HA.

pletely reversed by TMP when cultures were exposed to FUDR before infection, only partial reversal was obtained when TMP was added to cultures that had been treated with the analog for 12 hours after infection. These observations suggested that either the combined action of vaccinia virus and FUDR or an effect of the virus, not inhibited by FUDR, had caused irreversible damage to the cells. NP and LS antigens were normally synthesized well before infectious virus and HA (Loh and Payne, 1965). In those inhibited cultures treated with TMP at 12 hours p.i., a cell-damaging effect of the virus might have occurred after NP antigen was synthesized but before

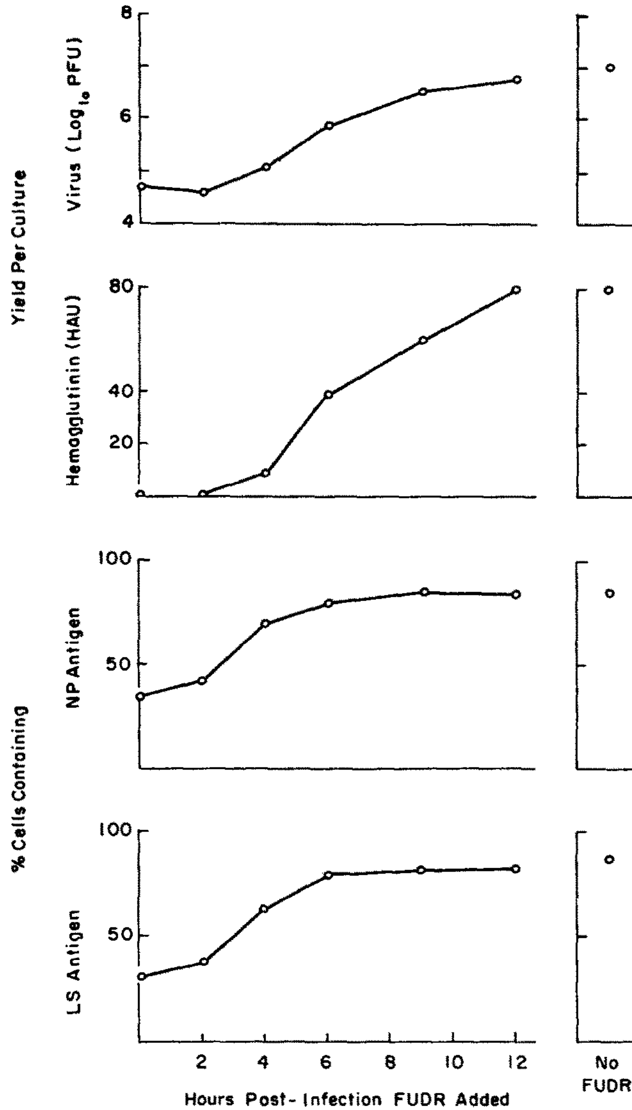

FIG. 2. The effect of FUDR added at the time of, and at various times after, infection on the production of vaccinia virus, vaccinial hemagglutinin, NP antigen, and LS antigen in HeLa cells. Replicate cultures were infected as described in Materials and Methods. FUDR to give a concentration of 10^{-6} *M* was added to the cultures at the indicated times. The proportion of cells staining with fluorescent antibody was determined using coverslips the cells on which were fixed at 12 hours p.i. Cultures were frozen at 24 hours p.i. for measurement of the yields of infectious virus and HA.

the synthesis of infectious virus and HA was complete.

Effect of FUDR Added at Different Times after Infection

In an attempt to relate the FUDR-sensitive reaction or reactions to time after infection, the synthesis of infectious virus and vaccinial antigens was examined in cultures to which the analog was added at various times after infection. The proportion of cells containing NP and LS antigen was determined at 12 hours p.i. and yields of infectious virus and HA were determined on samples taken at 24 hours p.i.

The results in Fig. 2 indicate that the inhibition of synthesis of infectious virus and viral antigens decreased progressively with increases in the time after infection at which FUDR was added. As was reported above, cultures receiving FUDR (10^{-6} M) at the time of infection contained at 24 hours p.i. the same amounts of infectious virus as did cultures after washing at the end of the 2-hour viral adsorption period. The yield of infectious virus was above this baseline in cultures receiving FUDR at 4 hours p.i. and approached a normal yield in cultures receiving FUDR between 9 and 10 hours p.i. As suggested by Salzman (1960), this curve probably represents the rate of synthesis of DNA destined to give rise to infectious virus. Thus the curve indicates that viral DNA synthesis began sometime before 4 hours p.i., which is in agreement with the previous observation (Loh and Payne, 1965) that in this cell–virus system acid insoluble thymidine-H^3 was first found in the cytoplasm of some infected cells at 2.5 hours p.i.

In cultures without FUDR, HA was first detected at 10–12 hours p.i. As seen in Fig. 2, HA was not detected at 24 hours p.i. when FUDR was added at 0 or 2 hours p.i. but was produced after FUDR was added at 4 hours p.i. That some viral DNA synthesis had occurred by 4 hours p.i. suggested that HA synthesis might depend on prior viral DNA synthesis. However, a similar curve of inhibition of HA synthesis, could also have resulted if a reaction, other than viral DNA synthesis, necessary for HA production was inhibited by FUDR.

In the experiment presented in Fig. 2, 35% of the cells produced NP and LS antigen after FUDR was added at 0 time. These cells had synthesized antigen in the absence of viral DNA synthesis as judged by their lack of production of infectious virus. In cultures receiving FUDR at any given time p.i., the percentage of cells containing NP antigen at 12 hours p.i. was similar to that containing LS antigen. This suggested that the ability of a cell to synthesize these two antigens was dependent on a common reaction which in some cells was inhibited by FUDR. In the absence of FUDR, LS and NP antigens were first detected in about 5% of the cells at 3 and 5 hours p.i., respectively, and were present in the majority of the infected cells at about 10 hours p.i. When FUDR was added to cultures at 6 hours p.i. or later the proportion of cells that produced antigen was the same as that found in cultures not treated with the analog. It seemed that either some FUDR-sensitive reaction was not completed in all the infected cells until 6 hours p.i. or the analog added at 6 hours p.i. did not inhibit antigen synthesis until some 4 hours later when all these cells had produced antigen.

DISCUSSION

It had been observed that many cells infected with vaccinia virus and treated with *p*-fluorophenylalanine were able to synthesize what appeared to be normal amounts of LS antigen in the absence of detectable viral DNA synthesis (Loh and Payne, 1965). It was thought that cells in which the synthesis of DNA and infectious virus had been inhibited by FUDR might also synthesize LS antigen, and possibly other viral antigens, in near normal amounts. Contrary to this expectation, it was found that in cultures treated with 10^{-6} M FUDR, the concentration required for maximal inhibition of synthesis of infectious virus, the proportion of cells synthesizing LS and NP antigen and the amounts of these antigens per cell was less than in cultures not treated with the analog. The yield of vaccinial hemagglutinin

in inhibited cultures at 24 hours p.i. was also markedly decreased. Nevertheless, it was apparent that some NP as well as LS antigen was synthesized under conditions where FUDR had markedly inhibited the synthesis of infectious virus and viral DNA. It was calculated that many of the inhibited cells containing LS and NP antigen produced no detectable infectious virus. This was in accord with observations made using *p*-fluorophenylalanine which had suggested that synthesis of viral DNA is not required for synthesis of viral antigens.

The results of the present investigation agree with those of Shatkin (1963), who described the formation of viral protein in HeLa cells treated with FUDR. In the latter experiments viral protein was quantitated by precipitation with antisera containing antibody against all viral proteins formed during virus synthesis. It was reported that cultures treated with 10^{-6} M FUDR produced less than normal amounts of viral antigen; the amount of antigen in inhibited cultures at 14 hours p.i. varied from 35 to 60% of that in cultures not treated with the analog.

Two reports (Easterbrook, 1963; Oda, 1963) have appeared suggesting that under some conditions nearly normal yields of vaccinial antigens can be obtained in the absence of viral DNA synthesis. Easterbrook (1963) has stated that fluorescent antibody staining and complement fixation tests at 24 hours p.i. revealed that the quantity of viral antigen in H.Ep.2 cells exposed to 10^{-6} M FUDR and 10^{-5} M uridine was indistinguishable from that in cells not so exposed. Also, electron microscopy of the FUDR-inhibited cells revealed large numbers of particles resembling "immature" virus, but no mature virions. Similarly, Oda (1963) used mitomycin C as an inhibitor of DNA synthesis and reported that in HeLa cells treated with this antibiotic multiplication of infectious virus was completely inhibited but formation of vaccinial hemagglutinin, complement-fixing antigen, and serum-blocking substance was little affected.

In the present experiments the degree of inhibition of synthesis of viral antigen was related to the concentration of FUDR and to the duration of exposure of the cells to the analog. Prolonged incubation of infected cells, but not noninfected cells, with FUDR produced irreversible damage to the cells as judged by their inability to produce normal yields of infectious virus. Although this damage may have resulted from effects of the virus not inhibited by FUDR, it was also possible that infected cells were more sensitive to damage by the analog. A similar combined effect of infection and treatment with FUDR also might have been responsible for the reversible inhibition of synthesis of viral antigen. Since the same pool of virus was used in all experiments and since the proportion of cells synthesizing viral antigen in the presence of FUDR varied between, but not within experiments, it was also possible that the physiologic state of the cells influenced their susceptibility to the analog.

No evidence was obtained to indicate that the inhibition of viral antigen synthesis was attributable to the conversion of FUDR to fluorouracil or its riboside derivatives which might have interfered with RNA synthesis. That TMP reversed the inhibition of antigen synthesis suggested that DNA synthesis was directly or indirectly involved. FUDR was effective in inhibiting the incorporation of deoxyuridine into nuclear DNA. Shatkin and Salzman (1963) have reported that thymidine is used preferentially for the synthesis of viral rather than nuclear DNA, and since no synthesis of viral DNA attributable to preformed thymidine derivatives was demonstrable in FUDR treated cultures, it might be assumed that the analog had effectively inhibited synthesis of nuclear DNA in those cells that synthesized antigen as well as those that did not. The evidence discussed above indicates that viral DNA synthesis was not required for antigen synthesis, and it is possible that inhibition of nuclear DNA synthesis, in the absence of viral DNA synthesis, had interfered with antigen synthesis in some cells but not in others. The physiologic state of the cell, perhaps as regards nuclear DNA synthesis, at the time FUDR was added

might have determined its ability to synthesize viral antigen in the absence of viral DNA synthesis.

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