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Fluorinated Pyrimidines

XXXVIII. The Incorporation of 5-Trifluoromethyl-2′-deoxyuridine into the Deoxyribonucleic Acid of Vaccinia Virus

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SUMMARY

A comparison has been made of the properties of purified vaccinia virions replicated in HeLa cells in the presence of thymidine or the powerfully antiviral 5-trifluoromethyl-2′-deoxyuridine (F_3TdR). The replacement of thymidine by the analogue in the DNA of the virions amounted to 1.4–9.8%, and such virions were noninfective. Sucrose density gradient centrifugation of the DNA isolated from purified virions revealed that the normal virions had a sedimentation coefficient of 70, whereas the analogue-containing DNA sedimented with *S*-values of 52 and 39. The presence of F_3TdR in the medium did not affect the process of uncoating of the infecting virions, but recoating of the viral DNA occurred to only 50% of normal. The morphology of vaccinia virions containing F_3TdR in their DNA was considerably different from normal.

INTRODUCTION

5-Trifluoromethyl-2′-deoxyuridine (trifluorothymidine) (1) is incorporated into the DNA of bacteriophage T4 (2), and, as shown in the accompanying paper (3), into the DNA of mammalian cells in culture. Moreover, it irreversibly inhibits thymidylate synthetase, after conversion to the corresponding 5′-monophosphate (4). F_3TdR[3] was shown by Kaufman and Heidelberger to inhibit herpes simplex keratitis in the rabbit's eye (5), and Kaufman subsequently showed that on a molar basis it is a considerably more potent inhibitor of viral production in this system than 5-bromo-2′-deoxyuridine and 5-iodo-2′-deoxyuridine (6). In this laboratory the effects of F_3TdR and other pyrimidine nucleoside analogues have been studied on various cells in culture (7), and on the replication of vaccinia virus in HeLa cells (8). It was shown (8) that F_3TdR inhibited viral replication at the lowest concentration of any of the analogues tested, and that its effect was prevented by the simultaneous addition of thymidine, but when thymidine was added 1 day after the time of infection in the presence of F_3TdR, vaccinia virus production was not rescued. This latter observation suggested that F_3TdR was incorporated into the DNA of the virus.

The incorporation of other pyrimidine

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[3] The abbreviations used are: F_3TdR, 5-trifluoromethyl-2′-deoxyuridine; BUdR, 5-bromo-2′-deoxyuridine; IUdR, 5-iodo-2′-deoxyuridine; TdR, thymidine; PFU, plaque-forming units.

nucleoside analogues into the DNA of animal viruses has been demonstrated in several laboratories. Easterbrook and Davern (9) showed that BUdR inhibited the replication of vaccinia virus in HeLa or KB cells and was incorporated into DNA; Prusoff *et al.* (10, 11), with IUdR and vaccinia virus, and Kaplan and his group (12, 13), with IUdR and pseudorabies virus, have made similar observations. McCrea and Lipman (14) showed by electron microscopy that vaccinia viral DNA containing IUdR was fragmented into short lengths as compared with the normal viral DNA. In the present paper we report studies on the incorporation of F_3TdR into the DNA of vaccinia virus replicated in HeLa cells.

MATERIALS AND METHODS

Cell culture and virus propagation. HeLa S3 cells (mycoplasm-free) were grown in shaker culture in minimal essential medium for suspension culture, 10% calf serum, 0.1% Pluronic F68, and antibiotics. For the maintenance medium for vaccinia (WR strain) replication, the calf serum was replaced by 0.1% bovine serum albumin fraction V. All media and sera were obtained from the Grand Island Biological Company. The conditions for the virus infection and plaque assays were exactly as described previously (8).

Purification of vaccinia virions. The purification of poxviruses from sonicated cytoplasmic fractions of cells by sucrose gradient centrifugation (15–17) and potassium tartrate gradient centrifugation (17, 18) has been described, and served as the basis for our procedure.

Infected HeLa cells were washed and suspended at a density of 2×10^7/ml in hypotonic medium (10^{-2} M Tris-HCl, pH 8–9; 10^{-2} M KCl; and 5×10^{-3} M $MgCl_2$) and allowed to swell for 10 min. They were then disrupted, using 10–15 strokes of a Dounce homogenizer (microscopic examination to control disruption), and centrifuged at $700 \times g$ for 10 min to remove the nuclei. The crude cytoplasmic fraction was sonicated in an MSE disintegrator for 1 min at 1.5 mamp and 125 V. The crude sonic extract (0.8 ml) was centrifuged through a 25–45% sucrose gradient (5×10^{-2} M Tris-HCl, pH 8.0) in the SW 25 rotor of a Spinco model L ultracentrifuge for 45 min at 15,000 rpm. The residual pellet was sonicated and centrifuged again. After the second centrifugation, 10-drop fractions were collected from the bottom of the tube and assayed for absorbance at 260 mμ, radioactivity, and plaque-forming units. Further purification was done by recentrifuging the virus band (fractions 6–11) two or three times. For large-scale isolations from the crude cytoplasmic sonic extract, the virus band was sedimented at 35% sucrose density, and the concentrate was then further purified by additional cycles of differential centrifugation in sucrose gradients. After purification of the vaccinia virions by sucrose gradient sedimentation, in some experiments potassium tartrate centrifugation was done in a 20–50% gradient (w/v) for 2.5 hr at 32,000 rpm.

Isolation of DNA. Method A: Cellular DNA was isolated from the nuclear fraction of the homogenate by the method of Kirby and Cook (19), and was treated with RNase; further details are given in the accompanying paper (3).

Method B: The DNA was isolated from purified virions as described by Pfau and McCrea (20). This procedure involves washing the virions with absolute ethanol and ethanol–ether (1:1), drying overnight at 40°, resuspending in 0.15 M NaCl and 0.015 M sodium citrate, making up to a final concentration of 2% with 2-mercaptoethanol, and stirring slowly for 12 hr in the cold room. Then 0.5 mg of Pronase (Calbiochem) was added, incubation with stirring was carried out at 37° for 24 hr, and the mixture was then centrifuged at $20,000 \times g$ (Sorvall) for 1 hr. The sediment could be treated as above to recover more DNA. Finally, the DNA was sedimented by centrifugation for 4 hr at 35,000 rpm in the SW 39 rotor of a Spinco model L ultracentrifuge.

Method C: The DNA was also isolated from purified virions by a modification of the method of Sarov and Becker (21). The pellet of virions was incubated for 2 hr with a mixture of 0.15 M NaCl, 0.015 M sodium citrate, 1% deoxycholate, 2% 2-methoxy-

ethanol, 10^{-3} M EDTA, and 0.2% sodium dodecyl sulfate. This mixture was then incubated overnight with stirring at 37° with 0.5 mg of Pronase, the sodium dodecyl sulfate concentration was increased to 2%, and the mixture was heated for 5 min at 60°, and layered over CsCl for density gradient centrifugation as described previously (3).

Sucrose gradient sedimentation of purified virion DNA. This was carried out exactly as described in procedure 2 (1) of Sarov and Becker (21). A similar preparation of virion DNA was used for alkaline sucrose sedimentation according to the method of McGrath and Williams (22). DNase treatment of the cytoplasmic sonic extract was carried out as described by Levitt and Becker (23).

Analytical methods. The concentration of DNA was determined by the method of Burton (24). The purified virions were diluted with 0.15 M NaCl and 0.015 M sodium citrate, and 0.1 ml was counted in 10 ml of "ANPO" solvent (3) in a Packard Tri-Carb liquid scintillation spectrometer. In some of the virion purifications, aliquots of the fractions were precipitated in the presence of albumin and counted on membrane filters (3). DNA obtained from CsCl centrifugation was dialyzed against 0.15 M NaCl and 0.015 M sodium citrate, and an aliquot was treated in the liquid scintillation vial with DNase and counted after the addition of "ANPO" mixture (3).

RESULTS

Purification of vaccinia virions. When the crude cytoplasmic sonic extract of HeLa cells that had been infected 24 hr previously with vaccinia virus in the presence of ^{3}H-TdR was centrifuged in a 25–45% sucrose gradient, the results shown in Fig. 1 were obtained. There were two major peaks of radioactivity. One, with a maximum in fraction 9, coincided exactly with the plaque-forming units; this peak was also found in a replicate experiment in which the sonic extract had been treated with DNase. Hence, this fraction contains infectious virions. The second peak of radioactivity, with its maximum in fraction 24, did not survive DNase treatment and was not infectious; thus, it was DNA. Further purification of the virion

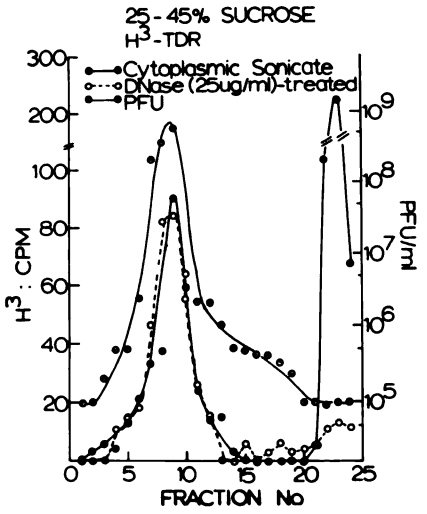

FIG. 1. *Sedimentation of vaccinia virus in a sucrose gradient*

The cells were infected with 10 PFU/cell at 5×10^5 cells/ml for 1 hr at 37°, the unadsorbed virions were washed off, and virus growth took place for 24 hr in the presence of 0.1 μCi/ml of ^{3}H-TdR. The cytoplasmic sonic extract (see the text) was centrifuged for 45 min at 15,000 rpm in the SW 39 rotor. Another 0.8-ml aliquot of the cytoplasmic sonic extract was digested with 25 μg/ml of DNase (Worthington) for 2 hr at 37° and centrifuged in the same way.

peak was carried out by potassium tartrate gradient centrifugation, as shown in Fig. 2. Here, the peak of ^{3}H, plaque-forming units, and A_{260} again coincided, the peak was sharper, and the free DNA had been eliminated.

When the crude cytoplasmic sonic extract from cells infected with vaccinia virus in the presence of F_3TdR-2-^{14}C was centrifuged through a sucrose gradient, the results shown in Fig. 3 were obtained. There were again major peaks of radioactivity and plaque-forming units at fraction 9 (peak I), and a noninfectious peak at fraction 24 (peak III) of free DNA. However, there was an additional small, slightly infective peak (II) at fraction 12 that was not found with the normal virus. Peaks I and II were recentrifuged through a sucrose gradient, with the results shown in Fig. 4. This confirms that peak II exists, although the original peak II was contaminated with free DNA. Thus, we found a difference between the centrifugal

characteristics of the normal vaccinia virions and those containing the analogue, F_3TdR.

Extent of synthesis of viral DNA from the labeled precursors. A number of experiments were carried out in which HeLa cells were infected with vaccinia virus in the presence of equimolar amounts (usually 10^{-6} M) of ^{3}H-TdR and ^{14}C-F_3TdR; the virions were purified by sucrose gradient sedimentation, and the amount of radioactivity was determined either in the purified virions or in the DNA isolated from them, or both. These experiments are summarized in Table 1. The results are calculated in terms of percentage replacement, a term that we define as follows: in the ^{3}H-TdR experiments, the

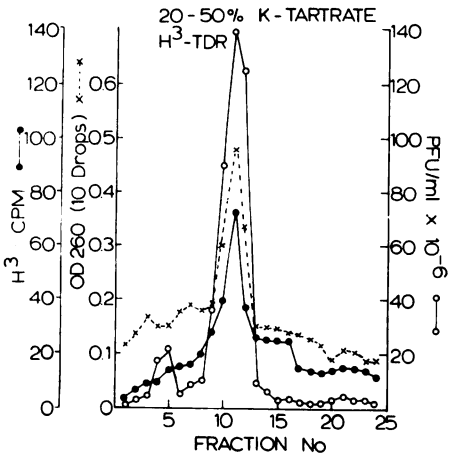

FIG. 2. *Sedimentation of vaccinia virus in a potassium tartrate gradient*

Viral replication took place in the presence of 0.025 μCi/ml of ^{3}H-TdR. The cytoplasmic sonic extract was centrifuged in 35% sucrose to concentrate the virions (see the text), which were resuspended and centrifuged in a 25–50% (w/v) gradient of potassium tartrate at 32,000 rpm for 2.5 hr at 4° in the SW 39 rotor.

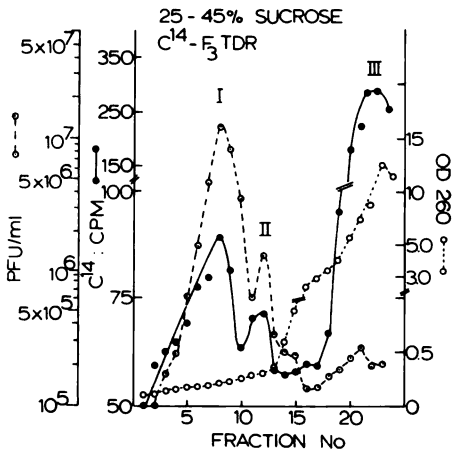

FIG. 3. *Sedimentation of vaccinia virus incubated with ^{14}C-F_3TdR*

The conditions of infection of the HeLa cells were the same as described for Fig. 1. Virus production took place for 24 hr in the presence of 10^{-6} M ^{14}C-F_3TdR. The conditions of isolation and centrifugation were identical with those described for Fig. 1.

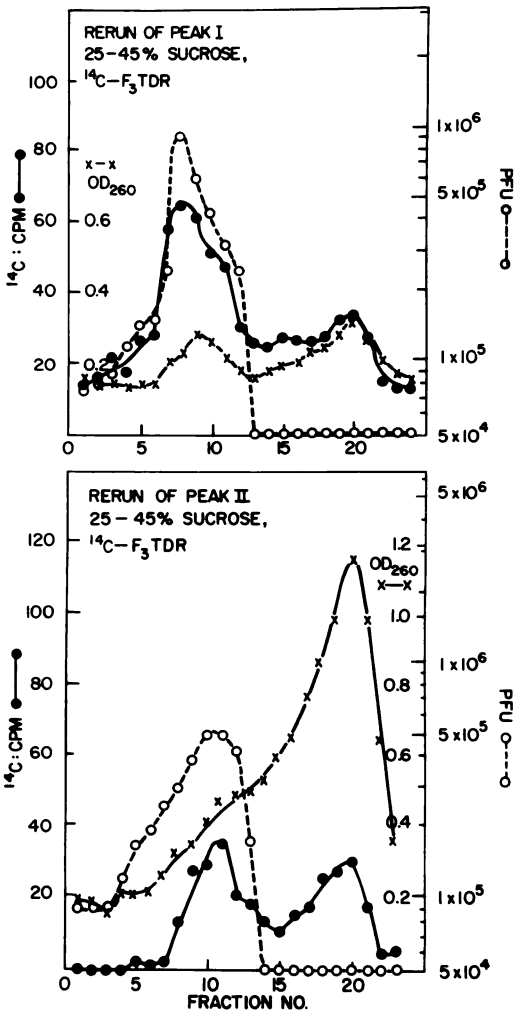

FIG. 4. *Resedimentation of peaks I and II of Fig. 3*

Peaks I and II of Fig. 3 were pelleted in the SW 25 rotor, sonicated briefly to suspend them, and centrifuged again through a sucrose gradient under the conditions described for Fig. 1.

TABLE 1

Extent of incorporation of TdR or replacement of TdR by F_3TdR in purified vaccinia viral DNA

Expt.	Precursor	Source of material	Specific activity			Replacement	Input multiplicity	Titer increase
			Nucleoside added	DNA	DNA dTMP (calculated)			
			dpm/μg	*dpm/μg*	*dpm/μg*	%		*-fold*
60	^{3}H-TdR, 10^{-6} M[a]	Virion[b]	9.15×10^6	2.41×10^3	7.90×10^3	0.09	10	
60	^{3}H-TdR, 10^{-6} M[a]	Virion DNA[c]	9.15×10^6	2.15×10^3	7.05×10^3	0.08	10	
62	^{14}C-F_3TdR, 10^{-6} M[a]	Virion	5.81×10^4	4.42×10^3	1.44×10^3	2.5	10	
62	^{14}C-F_3TdR, 10^{-6} M[a]	Virion DNA[c]	5.81×10^4	2.75×10^3	9.00×10^3	1.6	10	
53	^{14}C-F_3TdR, 10^{-6} M[a]	Virion	5.81×10^4	3.32×10^3	1.07×10^4	1.9	20	
74	^{3}H-UdR, 10^{-6} M[a]	Virion	9.20×10^5	7.58×10^3	2.56×10^4	2.9	5	
95	^{3}H-TdR, 10^{-6} M[a]	Virion	7.98×10^5	6.91×10^2	2.26×10^3	0.3	0.5	274
95	^{3}H-TdR, 10^{-6} M	Cell DNA[d]	7.98×10^5	1.14×10^2	4.10×10^2	0.05		
99	^{3}H-TdR, 10^{-6} M, +FUdR, 10^{-6} M[a]	Virion	1.07×10^6	1.79×10^4	5.58×10^4	5.3	0.43	120
99	^{3}H-TdR, 10^{-6} M, +FUdR, 10^{-6} M	Cell DNA	1.07×10^6	2.47×10^2	7.98×10^2	0.08		
100	^{14}C-F_3TdR, 0.29×10^{-6} M[a]	Virion	5.72×10^4	2.50×10^2	8.12×10^3	1.4	0.22	26.5
100	^{14}C-F_3TdR, 0.29×10^{-6} M	Cell DNA	5.72×10^4	8.6	27.8	0.05		
182	^{3}H-TdR, 10^{-6} M[e]	Virion	7.55×10^5	2.01×10^4	6.51×10^4	8.6	0.46	203
182	^{3}H-TdR, 10^{-6} M	Virion DNA[f]	7.55×10^5	1.78×10^4	5.75×10^4	7.6		
182	^{3}H-TdR, 10^{-6} M	Cell DNA	7.55×10^5	6.50×10^2	2.34×10^3	0.31		
183	^{14}C-F_3TdR, 10^{-6} M[e]	Virion	5.87×10^4	1.79×10^3	9.8	0.59	0.49	
183	^{14}C-F_3TdR, 10^{-6} M	Cell DNA	5.87×10^4	48.4	1.74×10^2	0.30		

[a] Labeled precursor was added at the time of infection.

[b] Total purified virions were counted in "ANPO" (3) and absorbance at 260 mμ was measured in dilute suspension.

[c] DNA was isolated from purified virions by method A.

[d] DNA was isolated from cells by method A.

[e] Labeled precursor was added 2 hr after infection.

[f] DNA was isolated from purified virions by method B.

percentage replacement is that percentage of the viral DNA thymidylic acid that was derived from the labeled thymidine added to the medium; and in the ^{14}C-F_3TdR experiments, the percentage replacement denotes the percentage of the dTMP in the viral DNA that has been replaced by the analogue. This is calculated as follows: in each case we have measured the specific activity of the labeled precursor added to the medium, and determined directly the specific activity of the DNA isolated from the purified virions. We know the base composition of purified vaccinia virions from Sarov and Becker (21), and hence we can calculate what the specific activity of the DNA dTMP would be if it were entirely derived from the added precursor, which at a level of 10^{-6} M would greatly exceed any intracellular pool of TdR or its metabolites. In the case of the purified virions, we determine the disintegrations per minute per A_{260} unit, and know (21) that 1 A_{260} unit contains 1.2×10^{10} virions, that the weight of the DNA per virion is 2.75×10^{-10} μg, and that the amount of DNA per absorbance unit of virions is 3.3 μg. Therefore, we can calculate the disintegrations per minute per microgram of virion DNA from the disintegrations per minute per A_{260} unit of purified virions.[4]

A number of conclusions can be drawn from the data presented in Table 1. (a) The amount of DNA dTMP derived from the TdR added to the medium is very small, amounting to replacements of 0.08%–8.6%, depending on the conditions. The reason for this small utilization will be reported elsewhere.[5] (b) The percentage replacements calculated from the determinations made on the purified virions are in good agreement with those found by direct assay of the DNA isolated from the purified virions. (c) The incorporation of ^{3}H-TdR and ^{14}C-F_3TdR into the vaccinia DNA was always considerably higher than that incorporated into the cellular DNA, which is to be expected from the fact that viral replication is carried out in resting cells and that the viral infection tends to shut off host cell DNA synthesis (25). (d) The replacement of viral DNA dTMP by F_3TdR was higher than the amount of TdR incorporated under comparable conditions, even though infective virus production (plaque-forming units) was inhibited. (e) As expected, the addition of FUdR stimulated the incorporation of TdR into the viral DNA, but the percentage replacement was still very low. (f) The incorporation of TdR and F_3TdR into the viral DNA was greater when the precursors were added 2 hr after infection rather than immediately after infection, probably as a result of the rapid degradation of the nucleoside precursors before DNA synthesis was initiated (which occurs after 2 hr). (g) The extent of F_3TdR replacement in the vaccinia viral DNA was considerably smaller than that reported for BUdR (9) and IUdR (10), although F_3TdR is more effective against vaccinia virus in our system than are BUdR and IUdR (8).

Infectivity of virions containing F_3TdR in their DNA. The infectivity of vaccinia virus replicated in media containing TdR and F_3TdR can be expressed as plaque-forming units per A_{260} unit of the virions purified by sucrose gradient centrifugation. The results of nine such experiments are shown in Table 2. In every case, the virions containing F_3TdR in their DNA were less than 6% as infective as the controls. However, since in

[4] This calculation for experiment 182 (Table 1) is illustrated as follows. The specific activity of the ^{3}H-TdR in the medium was 7.55×10^5 dpm/μg. The specific activity of the DNA isolated from the purified virions was 1.78×10^4 dpm/μg. The dTMP content of vaccinia DNA is 0.31 μg/μg of DNA (21). Thus the specific activity of the DNA dTMP is $(1.78 \times 10^4)/0.31 = 5.75 \times 10^4$ dpm/μg of dTMP in the DNA. Thus,

Percentage replacement

$$= \frac{5.75 \times 10^4 \times 10^2}{7.55 \times 10^5} = 7.6$$

In the case of the purified virions, the specific activity was 2.54×10^3 dpm/0.038 A_{260}. It is known (21) that 1 A_{260} unit of vaccinia virions contains 3.3 μg of DNA. Hence, this sample contained $0.038 \times 3.3 = 0.125$ μg of DNA. The specific activity of the DNA, calculated from counting the virions, was $(2.54 \times 10^3)/0.125 = 2.01 \times 10^4$ dpm/μg of DNA. The rest of the calculation is as above.

[5] Y. Fujiwara, T. Oki and C. Heidelberger, manuscript in preparation.

TABLE 2

Infectivity of normal and F_3TdR-containing purified virions

Expt.	Precursor	A_{260}/ml	Replacement	PFU/ml	PFU/A_{260}	Percentage of controls
			%			%
32	TdR	0.08		1.4×10^8	1.8×10^9	
40	F_3TdR, peak I[a]	0.22		1.1×10^7	5.5×10^7	3.1
40	F_3TdR, peak I rerun	0.13		9.0×10^6	7.2×10^7	4.0
40	F_3TdR, peak II[a]	0.33		1.2×10^6	4.0×10^6	0.22
40	F_3TdR, peak II rerun	0.22		5.0×10^5	2.2×10^6	0.12
99	TdR	0.33		5.0×10^8	1.54×10^9	
100	F_3TdR	0.94	1.4	7.9×10^7	8.37×10^7	5.4
182	TdR	0.50		1.2×10^8	2.4×10^8	
183	F_3TdR	0.31	9.8	6.1×10^5	2.0×10^6	0.83

[a] These refer to the peaks of Fig. 3.

the purification of the virions there is no difference in sedimentation between the normal virions and those containing the analogue, the low infectivity in the F_3TdR experiments could be accounted for entirely by the input virus. Thus, to a first approximation, we can say that vaccinia virions containing even as little as 1.4% replacement of DNA thymidine by F_3TdR are not infective. Peak II (Fig. 3) from experiment 40 was also essentially noninfective, but there is no normal counterpart to compare it with.

Sucrose gradient sedimentation of DNA of purified vaccinia virions. When the DNA isolated from purified vaccinia virions that had replicated in the presence of ^{3}H-TdR or ^{14}C-F_3TdR was centrifuged in a sucrose gradient, the results shown in Fig. 5 were obtained. It can be seen that the sizes of the two DNA samples were quite different. We have taken the data of Sarov and Becker (21), and on the basis of their standard phage T2 DNA having a sedimentation coefficient of 65 (26), have calculated that their *S*-value of vaccinia DNA would be 68. Our data in Fig. 5A are calculated by the method of McGrath and Williams (22) to give a value of 73 S. These values are considered to be in good agreement, and so we have taken the value of 70 S as the standard for normal vaccinia DNA. In Fig. 5A–C, the *S*-values were calculated by the methods of both McGrath and Williams (22) and Burgi and Hershey (27), and they always agreed to within 10%. Whereas the normal vaccinia

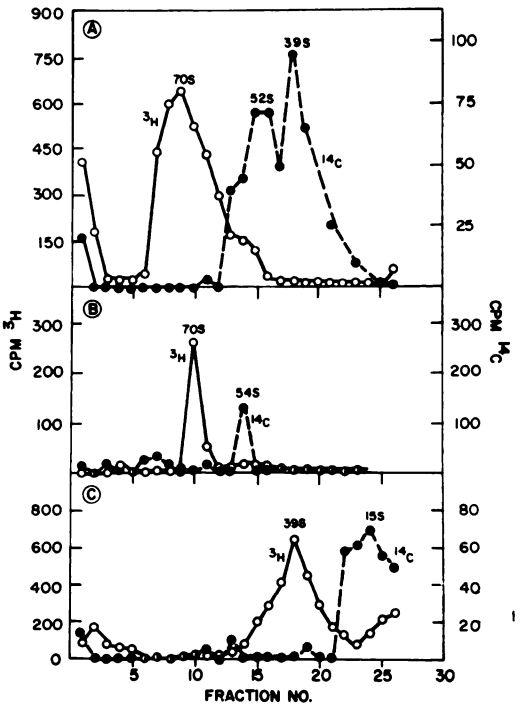

FIG. 5. *Sedimentation in a 5–20% sucrose gradient of DNA isolated from purified virions*

The virus was replicated in the presence of 10^{-6} M ^{3}H-TdR or 10^{-6} M ^{14}C-F_3TdR. The DNAs were combined and centrifuged in the SW 39 rotor of a Spinco model L ultracentrifuge for 4 hr at 25,000 rpm at 10°. A. Sedimentation in neutral sucrose of DNA from vaccinia virions. B. Resedimentation of the 70 S and 52 S peaks from A in neutral sucrose. C. Alkaline sucrose sedimentation of the DNA from vaccinia virions.

DNA had an *S*-value of 70, corresponding to a molecular weight of about 150 × 10^6 (21), the virions containing F_3TdR in their DNA sedimented with values of 52 and 39 S. On resedimentation of the 70 and 52 S peaks (Fig. 5B), essentially the same *S*-values were obtained. The DNA with an *S*-value of 52 corresponds to a molecular weight of 87 × 10^6, or about one-half that of normal vaccinia DNA (21). On alkaline sucrose sedimentation (Fig. 5C), as expected, the sedimentation of the two DNA samples was slower. Therefore, the DNA of the virions containing the analogue is smaller in size than normal, which may be one factor in explaining the lack of infectivity of these virions.

Cesium chloride density gradient centrifugation. The DNA samples obtained from the same experiments (Nos. 182 and 183) described for the sucrose sedimentations were combined, and preparative cesium chloride centrifugation was carried out as shown in Fig. 6. The DNA containing the F_3TdR had a slightly higher buoyant density than did the normal vaccinia DNA, which was calculated (28) as 11.1% replacement, in good agreement with the value of 9.8% replacement obtained by measurement of the radioactivity (Table 1).

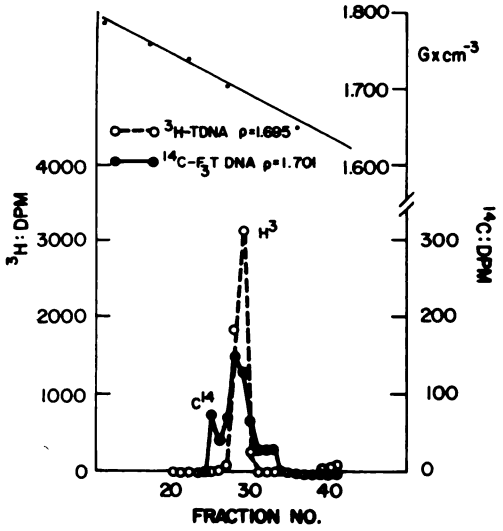

FIG. 6. *Cesium chloride gradient centrifugation of vaccinia viral DNA*

The mixed DNA samples from Fig. 5 were also subjected to CsCl equilibrium gradient centrifugation at 33,000 rpm for 68 hr at 25° in the SW 39 rotor. The densities were determined by pycnometry.

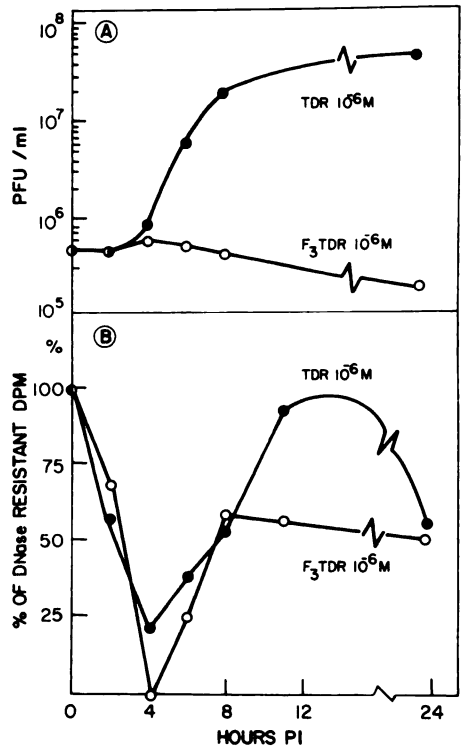

FIG. 7. *Study of the uncoating and recoating of vaccinia virus*

Purified virions that had been labeled with ^{3}H-TdR were used to infect HeLa cells. At various times thereafter the number of plaque-forming units per milliliter (A) was measured in cells treated with 10^{-6} M TdR and 10^{-6} M F_3TdR. The same cytoplasmic homogenates were incubated with DNase (100 μg/ml) and 0.01 M Mg^{++} for 60 min at 37° (23). They were then precipitated with cold 5% trichloracetic acid and filtered. This DNase-stable radioactivity was compared with that of aliquots that were not treated with DNase. The results are expressed as percentage of DNase-resistant disintegrations per minute (B). PI, postinfection.

Effect of F_3TdR on uncoating and recoating of vaccinia virus. Purified vaccinia virions that had been labeled with ^{3}H-TdR were used to infect cells in the presence of 10^{-6} M TdR or 10^{-6} M F_3TdR. As shown in Fig. 7A, there was extensive virus production with TdR in the medium, but essentially com-

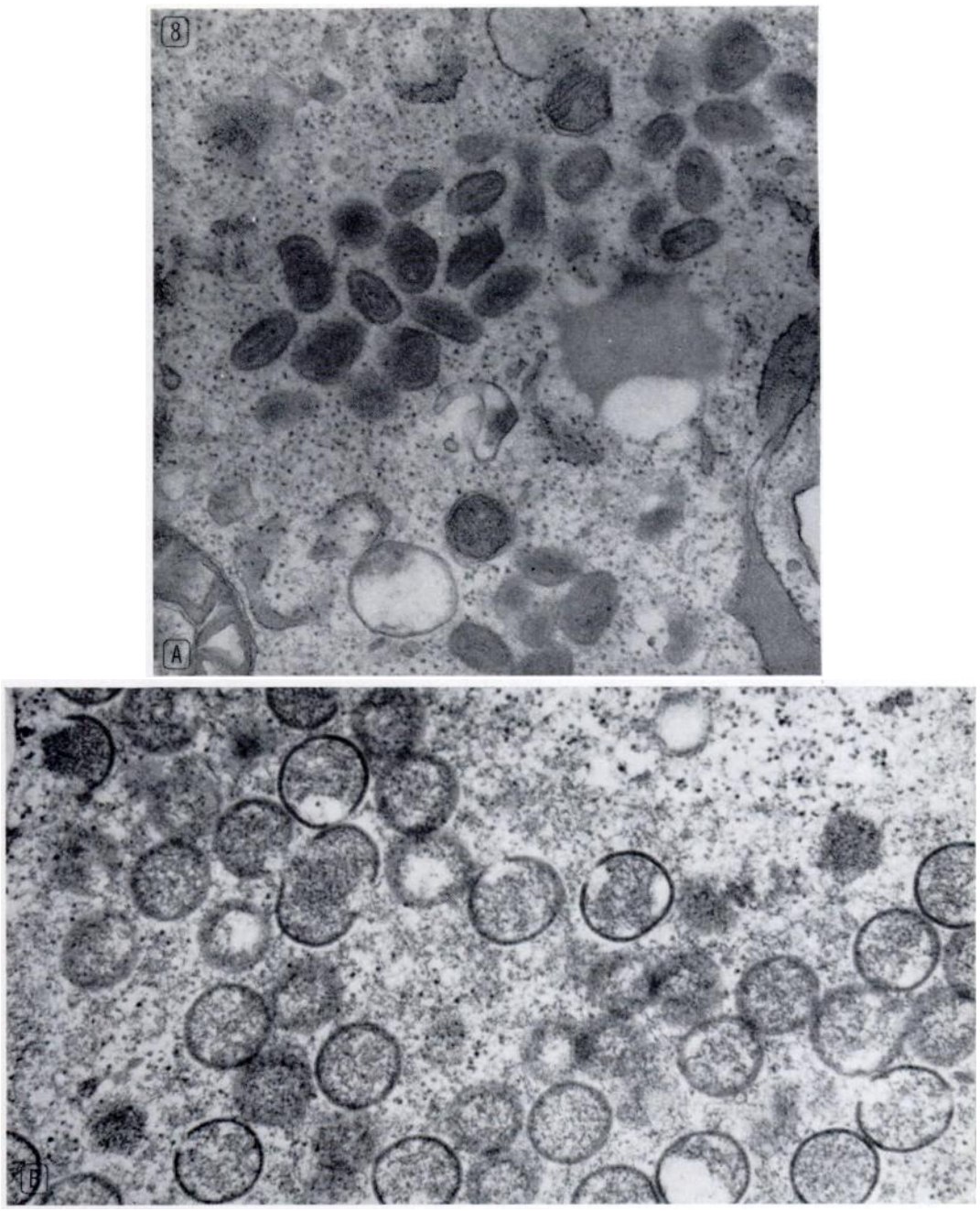

FIG. 8. *Thin sections prepared from HeLa cells 24 hr after infection with vaccinia virus in the presence of 10^{-6} M TdR (A) or 10^{-6} M F_3TdR (B)*

The cells were fixed with 1% glutaraldehyde, postfixed with osmium tetroxide, dehydrated with ethanol from 25% to absolute, and embedded in Epon Araldite. The thin sections were cut on a Porter-Blum ultramicrotome, stained with lead citrate, and examined on a Hitachi HU-11B electron microscope at a final magnification of 60,000. We are greatly indebted to Mr. George Pridmore and Professor James Perdue of this laboratory for taking these electron micrographs.

plete inhibition of plaque-forming units in the F_3TdR group. In the same experiment, the cytoplasmic sonic extracts were subjected to treatment with (23) and without DNase, and the percentage of DNase-resistant, trichloracetic acid-precipitated DNA was counted. It can be seen from Fig. 7B that the presence of F_3TdR in the medium did not affect the normal process of uncoating of the infecting virions (0–4 hr). However, the recoating of the viral DNA only occurred to a 50% extent in the F_3TdR group.

Morphology of normal and F_3TdR-containing virions. Electron micrographs of infected cells containing normal vaccinia virus and those found in infected cells treated with F_3TdR are shown in Fig. 8A and B, respectively. The normal cells contain mature virions with an oblong shape 0.25 × 0.13 μ and considerable internal structure. The treated cells contain particles of considerably different morphology, consisting of circular (0.29-μ) bodies with rather thick walls and little internal structure. These resemble very closely the particles described by Easterbrook and Davern (9) that were seen in vaccinia-infected cells that had been treated with BUdR, an analogue that is also incorporated into viral DNA. An occasional particle similar to those shown in Fig. 8B was seen in the normally infected cells, and a few normal virions could be found in the F_3TdR-treated cells. It is not now known whether the particles seen in the F_3TdR-treated cells represent an incomplete form of virion that is on the normal route to completion. Further studies may clarify this point.

DISCUSSION

5-Trifluoromethyl-2′-deoxyuridine on a molar basis is the most active compound known against DNA viruses (6), and consequently its mode of action is of interest. In a preliminary study from this laboratory (8) it was reported that F_3TdR inhibition could not be rescued by thymidine after 1 day, and it was suggested that this irreversibility might be a consequence of the incorporation of the analogue into viral DNA, rendering it somehow ineffective. In the present work it has been demonstrated that F_3TdR is incorporated into the DNA of purified vaccinia virions, but to a much lesser extent than has been found for BUdR (9) and IUdR (10, 11). Nevertheless, virions containing as little as 1.4% replacement of DNA thymidine by F_3TdR were noninfective.

Sucrose density gradient sedimentation of the DNA isolated from normal and F_3TdR-containing virions has demonstrated that the latter DNA is smaller in size than normal. This observation is in agreement with our finding that in synchronized L5178Y cells the assembly of small units of DNA into larger molecules is inhibited when the synthesis occurs with incorporation of F_3TdR (3). Whether this inhibition represents chain termination by the analogue or some other mechanism remains to be determined.

The selective chemotherapeutic effect of F_3TdR against the virus as compared with the host cells may be explained by the fact that it is incorporated to a considerably greater extent into viral than into cellular DNA under conditions of infection.

It seems clear at this point that the incorporation of F_3TdR into vaccinia viral DNA is responsible for the antiviral activity of the drug. There appear to be at least three mechanisms whereby this incorporation into DNA could prevent viral infectivity and replication: (a) the incorporation of the analogue prevents the replication of viral DNA, and the smaller size of the virion DNA suggests that this may be so; (b) the incorporation of the analogue causes the formation of faulty early messengers; or (c) the incorporation of the analogue causes the formation of faulty late messengers and, hence, faulty structural proteins, which might account for the altered morphology and incomplete recoating of the analogue-containing virions. Work is in progress in this laboratory designed to distinguish among these possible mechanisms of antiviral activity of F_3TdR.

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