Ribonucleotides in Infectious Bovine Rhinotracheitis Virus DNA

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SUMMARY

Infectious bovine rhinotracheitis (IBR) virus was grown in the presence of 5-3H-uridine in a continuous line of bovine kidney cells. 5-3H-uridine was found to be associated with viral nucleocapsids. Furthermore, purification of the viral nucleic acid present in nucleocapsids illustrated that 5-3H-uridine was part of the viral nucleic acid. Purification of viral DNA from infected cells also indicated that 5-3H-uridine was associated with viral nucleic acid possibly as ribonucleotides. The label was identified as RNA by measuring its susceptibility to RNase and analysis of the bases. Short pulses with 5-3H-uridine, resulted in labelled nucleic acid which was extremely sensitive to RNase and alkali but resistant to DNase. Nucleotide analysis indicated that after short pulses all the radioactivity was associated with the base uracil whereas upon longer labelling periods a large percentage of the label was associated with cytosine. However even if viral DNA was isolated from nucleocapsids there was still some radioactivity associated with uracil. Sedimentation of heat denatured 5-3H-uridine label viral nucleic acid in Cs₅SO₄ indicated that the label sedimented at a density of single stranded DNA suggesting that the ribonucleotides are covalently linked to the viral DNA.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) virus, a member of the herpesvirus family, consists of a single intact unsegmented double stranded DNA genome (Gentry & Randall, 1973). The genome of all herpesviruses so far investigated fragments into smaller pieces upon denaturation in alkali (Nonoyama & Pagano, 1971; Frenkel & Roizman, 1972; Mosmann & Hudson, 1973). Recently the presence of RNA-like material has been demonstrated in mature herpes simplex virus DNA (Hirsch & Vonka, 1974) and it has been suggested that this RNA-like material may account for the alkaline labile regions within the genome.

These RNA regions may arise as a result of the participation of RNA in the initiation of DNA synthesis (Sugino, Hirose & Okazaki, 1972; Wickner et al. 1972). This possibility is supported by the observation that newly synthesized DNA of M-13 (Wickner et al. 1972), φ X 174 (Schekman et al. 1972), Escherichia coli (Sugino et al. 1972), polyoma (Magnusson et al. 1973) and herpes simplex (Biswal, Murray & Benyesh-Melnick, 1974; Murray & Biswal, 1974) viruses all contain RNA covalently linked to their DNA. Furthermore, newly synthesized herpes simplex virus DNA was shown to contain a much larger amount of RNA-like material than did virus DNA extracted after longer labelling periods. These observations suggest that RNA is involved in herpes virus DNA replication and in addition that this RNA is not removed completely after replication is complete.
Our results demonstrate that RNA is also present in the IBR virus genome and such RNA is present to a greater extent in newly synthesized DNA as compared to mature DNA. These observations support the hypothesis that RNA is involved in IBR DNA synthesis and may also account for fragmentation of the genome upon treatment with alkali.

METHODS

Solutions and reagents. Virus buffer (VB) consisted of 0.15 M-NaCl and 0.02 M-tris-hydrochloride, pH 7.5. Neutral DNA buffer (NDB) consisted of 1 M-NaCl, 0.001 M-EDTA, and 0.05 M-tris-hydrochloride, pH 7.5. Reticulocyte standard buffer (RSB) consisted of 0.01 M-NaCl, 0.001 M-MgCl₂ and 0.01 M-tris-hydrochloride, pH 7.5. Standard saline citrate (SSC) consisted of 0.15 M-NaCl and 0.015 M-trisodium citrate. TNE buffer consisted of 0.1 M-NaCl, 0.001 M-EDTA and 0.01 M-tris-hydrochloride, pH 7.5. TNM buffer consisted of 0.01 M-NaCl, 0.001 M-MgCl₂ and 0.01 M-tris-hydrochloride, pH 7.5. The isotopes used were 5-³H-uridine (25 Ci/mmol; New England Nuclear, Dorval, Quebec) and methyl-³H-thymidine (5 Ci/mmol; Amersham Searle, Oakville, Ontario). Ribonuclease A 5 times crystallized and ribonuclease T₁ were obtained from Sigma (St Louis, Mo). These enzymes were heated for 10 min in TNM buffer at 80 °C prior to use. Deoxyribonuclease (RNase free) was obtained from Worthington Biochemicals (Freehold, N.J.). Caesium chloride and caesium sulphate were obtained from Sigma (St Louis, Mo.).

Cells. Madin Darby bovine kidney (MDBK) cells were cultured in Eagle’s minimal essential medium (MEM). Each litre was supplemented with 2 mmol glutamine, 10 ml non-essential amino acids (Gibco No. 114), 50 mg gentamycin, 25 g of sodium bicarbonate and 10 % foetal calf serum (FCS; Gibco No. 614).

Virus. Strain P8-2 of IBR virus was kindly provided by Dr J. R. Saunders. This virus received 2 passages through primary foetal bovine kidney cell monolayers before preparation of stock virus. Stock virus was prepared in MDBK cells as described previously (Rouse & Babiuk, 1974).

Infection and labelling of virus nucleic acid. Confluent monolayers (150 mm glass Petri dishes) of MDBK cells were infected with 5 p.f.u. of virus per cell in a volume of 0.5 ml. The virus was allowed to adsorb for 90 min with occasional gentle rocking. After 90 min the cell monolayer was washed and refed with MEM + 4 % FCS. Zero time was considered to be the end of the adsorption period. Six hours post-infection (p.i.), 5-³H-uridine (50 μCi/ml) or methyl-³H-thymidine (10 μCi/ml) was added.

Preparation of nucleocapsids. Nucleocapsids were prepared by the method of Kieff, Bachenheimer & Roizman (1971). At 18 to 20 h p.i. the infected monolayers were washed in Puck’s solution G (PS), the cells were scraped from the monolayer and pelleted at 1000 g for 5 min. The pelleted cells were resuspended and allowed to swell for 10 min in ice cold RSB containing 0.5 % Nonidet P-40 (NP 40; Sigma, St Louis, Mo.) after which time they were homogenized by 10 gentle strokes of a tight fitting Dounce homogenizer. The resulting nuclei and cytoplasmic extract were examined under phase contrast microscopy to determine the number of disrupted cells. In all cases over 85 % of the cells were disrupted to yield free nuclei. Nuclei were removed by centrifuging, at 1000 g for 10 min, and the resulting cytoplasmic extract (2 ml) was layered on a 36 ml sucrose gradient (10 to 50 %, w/w, in VB), gradients were centrifuged at 20000 rev/min for 80 min at 4 °C in an IEC type SB-110 rotor. Fractions of 45 drops each were collected from the bottom of the tube. Radioactivity and optical density of each fraction was monitored. The fractions containing virus were pooled, resuspended in virus buffer and pelleted at 20000 rev/min (3 h, in an IEC Type SB-283). In
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In some instances, the viral nucleocapsids were treated with DNase (20 μg/ml) in TNM buffer or with RNase (20 μg/ml RNase A and 20 units RNase T₁) in TNM buffer for 1 h at 37 °C followed by re-pelleting under conditions described above.

**DNA extraction from nucleocapsids.** Pelleted nucleocapsids were dissolved in neutral DNA buffer containing 0.5% (w/v) sodium dodecyl sulphate and 2% (w/v) sodium N-lauryl sarcosinate and gently rolled with phenol. Following removal of the phenol phase, the aqueous phase was rolled with chloroform-isooamylalcohol (4%, v/v) until the aqueous phase containing DNA was clear. The DNA sample was gently layered onto a 11.5 ml sucrose gradient (10 to 30%, w/w, in NDB) and centrifuged at 40000 rev/min for 4 h at 20 °C (in an IEC Type SB-283 rotor). Fractions were collected from the bottom and assayed for radioactivity. The fractions containing viral DNA were pooled and dialysed extensively against 0.5 × SSC. Solid CsCl or Cs₂SO₄ was added to give a density of 1.710 g/ml or 1.550 g/ml, respectively, and centrifuged to equilibrium at 35000 rev/min for 3 days at 20 °C (in an IEC Type SB-405 rotor). Radioactivity and the refractive index of the various fractions was monitored.

**DNA extraction from infected cells.** At various times p.i., the infected cells were washed in TNE buffer and lysed in 0.5% sodium dodecyl sulphate in TNE buffer followed by treatment with phenol. The aqueous phase was dialysed against 0.5 × SSC. Solid CsCl was added to give a density of 1.710 g/ml. The mixture was placed in a polyallomer tube and centrifuged at 30000 rev/min for 3 days at 20 °C (in an IEC Type SB405 rotor). Four drop fractions were collected from the bottom of the tube and analysed for optical density, radioactivity and refractive index. Fractions containing radioactivity and at a density in the region of 1.725 to 1.730 g/ml were pooled and re-centrifuged in a similar CsCl gradient. The presence of contaminating host DNA in the virus DNA fraction was monitored using an analytical ultracentrifuge (Beckman Model E).

**Nucleotide analysis.** 5-³H-uridine or methyl-³H-thymidine labelled virus DNA was mixed with 1 mg of calf thymus DNA and hydrolysed by the addition of 1 ml of 88% formic acid (Bendich, 1957). The solution of DNA and formic acid was placed in Pyrex glass tubes which were sealed under vacuum and heated for 30 min at 175 °C. The hydrolysed solutions were dried under vacuum over KOH, diluted in 1 N-HCl and chromatographed on Cellulose MN 300 chromatography paper (Brinkman Instruments, Rexdale, Ontario). The solvent consisted of isopropanol : HCl : H₂O (70 : 10 : 20). The chromatograms were dried, cut into strips and counted to determine the distribution of radioactivity.

**RESULTS**

**Incorporation of 5-³H-uridine into IBR virus nucleic acid**

The genome of all herpesviruses, including IBR, studied to date fragments upon treatment with alkali (L. A. Babiuk, unpublished results; Nonoyama & Pagano, 1971; Frenkel & Roizman, 1972; Mosmann & Hudson, 1973). Whether these alkaline labile regions were due to the presence of RNA in the genome was investigated. 5-³H-uridine was added to IBR infected cells 6 h p.i. and at 20 h p.i. the cells were disrupted and the nucleocapsids were analysed. Although the amount of radioactivity associated with the nucleocapsids was considerably lower with the 5-³H-uridine labelled nucleocapsids (Fig. 1a) compared to methyl-³H-thymidine labelled nucleocapsids (Fig. 1b), the radioactive peaks were essentially in identical positions indicating that the particles containing the radioactivity were approximately the same size. Treatment of the nucleocapsids with RNase or DNase prior to sedimentation in a velocity sucrose gradient did not alter the sedimentation rate of 5-³H-uridine labelled nucleocapsids, suggesting that the label was not present as a fragment of...
Fig. 1. Sedimentation of nucleocapsids in neutral sucrose gradients. IBR virus infected MDBK cells were labelled from 6 to 20 h p.i. with either 50 μCi 5-3H-uridine/ml or 10 μCi of methyl-3H-thymidine/ml. The nucleocapsids from 2 x 10⁶ cells were extracted and centrifuged through a 10 to 50 % sucrose gradient for 80 min at 20,000 rev/min in a IEC SB-110 rotor. Fractions (1.5 ml) were collected from the bottom of the gradient and samples (100 μl in the case of 5-3H-uridine and 50 μl in the case of methyl-3H-thymidine) were assayed for radioactivity. (a) Gradient containing 5-3H-uridine labelled nucleocapsids. (b) Gradient containing methyl-3H-thymidine labelled nucleocapsids. The bars indicate the fractions of each gradient, containing nucleocapsids, which were pooled for isolation of IBR DNA.

RNA attached to the exterior of the nucleocapsids but that the label was associated with the virion such that it was inaccessible to nuclease digestion. Furthermore, the amount of acid insoluble radioactivity of labelled nucleocapsids was not diminished following RNase or DNase treatment, indicating that all the label was in the virion.

The fractions containing nucleocapsids were pooled and pelleted. The nucleic acid was gently extracted and layered on to a neutral sucrose gradient. Analysis of the gradient (Fig. 2) indicated that the nucleic acid labelled with 5-3H-uridine sedimented at the same rate in one major peak as did nucleic acid labelled with methyl-3H-thymidine. Furthermore, this nucleic acid had a density of approx. 1.720 to 1.730 g/ml in CsCl – a density characteristic of IBR virus DNA (Fig. 3; Graham et al. 1972).

In a series of experiments, the infected cells were pulsed for short periods (20 min, 2 h or 4 h) with 5-3H-uridine or methyl-3H-thymidine after which time the intracellular virus nucleic acid was extracted and sedimented to equilibrium in a CsCl gradient. Fig. 4(a) illustrates that viral DNA could be partially purified from contaminating host cell DNA. The majority of the radioactivity was associated with the heavier IBR virus DNA. Analysis of this same preparation by analytical ultracentrifugation confirmed the presence of IBR DNA in the preparation (Insert, Fig. 4a). Fractions (33-39), banding at a density of 1.725 to 1.730 g/ml, were pooled and recentrifuged to equilibrium in a second CsCl gradient. Fig. 4(b)
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Fig. 2. Sedimentation of IBR virus DNA in neutral sucrose gradients. Labelled DNA was extracted from nucleocapsids and layered onto a neutral 10 to 30 % sucrose gradient. Centrifugation was for 4 h at 40000 rev/min in a IEC SB283 rotor. Fractions (0·6 ml) were collected from the bottom of the gradient and samples (20 µl in the case of methyl-3H-thymidine and 50 µl in the case of uridine) were assayed for radioactivity. (a) DNA extracted from methyl-3H-thymidine labelled nucleocapsids, 2·5 × 10⁴ ct/min layered onto gradient. Recovery 83 %. (b) DNA extracted from 5-3H-uridine labelled nucleocapsids, 7 × 10⁴ ct/min layered onto gradient. Recovery 91 %.

Fig. 3. Equilibrium centrifugation of IBR DNA isolated from viral nucleocapsids labelled with 3H-uridine (1·1 × 10⁵ ct/min). DNA was obtained from the fractions indicated by the bars in Fig. 1 and 2. This DNA was dialysed against 0·5 x SSC, solid CsCl was added to a density of 1·710 g/ml and centrifuged to equilibrium at 35000 rev/min in a IEC SB405 rotor at 20 °C. Four drop fractions were collected from the bottom of the tube directly into a scintillation vial and the entire sample was counted in a liquid scintillation counter. The bouyant density of approx. every fifth fraction was measured.
Fig. 4. Equilibrium sedimentation of DNA extracted from IBR infected cells. (a) 6 h p.i., cultures were pulsed for 8 h with 10 μCi/ml of methyl-3H-thymidine. The DNA from 4 × 10⁷ cells was extracted (see Methods) and a sample was centrifuged to equilibrium in a Beckman Model E analytical ultracentrifuge to determine whether any viral DNA was present. Insert in (a). Another sample (6.1 × 10⁶ cts/min) was centrifuged to equilibrium (72 h) at 30000 rev/min in CsCl (1.710 g/ml) in a IEC SB405 rotor at 20 °C. Fractions (4 drops) were collected from the bottom of the tube and analysed for buoyant density (■–■) and the radioactivity of a sample (10 μl) of each fraction was then determined. Fractions 33–39 from the gradient illustrated above were pooled. A sample of the above was once again monitored in an analytical centrifuge to determine the quantity of host cell DNA still present after 1 CsCl centrifugation. (Insert in b.) (b) The balance of fractions 33–39 were centrifuged to equilibrium in a SB405 rotor and once again monitored for buoyant density (□–□) and radioactivity (×–×) as described previously. A sample of fractions 32–38 were once again monitored in the analytical ultracentrifuge and found to be free of contaminating host cell DNA. The figure is representative of numerous gradients performed following labelling for various periods of time with either 5-3H-uridine or methyl-3H thymidine.

insert, illustrates that the percentage of host cell DNA was considerably reduced from the original starting material. Once again the fractions corresponding to 1.725 g/ml were collected and analysed by analytical ultracentrifugation. These fractions contained radioactivity when labelled with either 5-3H-uridine or methyl-3H-thymidine but were free of any detectable host cell DNA.

Taken together the above affirmations all suggest that RNA may possibly be associated with both newly synthesized intracellular virus nucleic acid as well as with mature nucleic acid present in nucleocapsids.

Presence of ribonucleotides in purified IBR DNA

The presence of radioactivity in IBR DNA following labelling with 5-3H-uridine could be due to the conversion of uridine to cytidine which could subsequently be incorporated into
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Table 1. Effect of nucleases and alkali on infectious bovine rhinotracheitis virus DNA*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCA precipitable radioactivity†</th>
<th>20 min (3H-uridine)</th>
<th>2 h (3H-uridine)</th>
<th>2 h (3H-thymidine)</th>
<th>4 h (3H-uridine)</th>
<th>20 h (3H-uridine)</th>
<th>20 h (3H-thymidine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>642</td>
<td>502</td>
<td>2141</td>
<td>1914</td>
<td>1719</td>
<td>7781</td>
<td></td>
</tr>
<tr>
<td>RNase‡</td>
<td>52</td>
<td>120</td>
<td>146</td>
<td>310</td>
<td>182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNase §</td>
<td>510</td>
<td>406</td>
<td>146</td>
<td>310</td>
<td>182</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td></td>
<td></td>
<td>31</td>
<td>86</td>
<td>2047</td>
<td>911</td>
<td>7657</td>
</tr>
</tbody>
</table>

* IBR infected MDBK cells were labelled for various times, beginning at 6 h p.i., with either 50 μCi/ml of 5-3H-uridine or 10 μCi/ml of methyl-3H-thymidine. The DNA was extracted and the IBR DNA was separated from host cell DNA by centrifuging twice to equilibrium in CsCl.
† Each sample was precipitated with 10% TCA. Counts are expressed as the amount of radioactivity retained on glass filter papers.
‡ RNase A (20 μg/ml) and RNase T1 (20 units/ml) in TNM buffer for 60 min.
§ DNase (20 μg/ml) in TNM buffer for 60 min.
|| 100 °C for 10 min in 0.5 M-NaOH.

Table 2. Radioactivity in bases of 5-3H-uridine labelled infectious bovine rhinotracheitis virus DNA

<table>
<thead>
<tr>
<th>Base</th>
<th>Pulse time</th>
<th>20 min</th>
<th>2 h</th>
<th>4 hr</th>
<th>20 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosine</td>
<td>48†</td>
<td>306</td>
<td>410</td>
<td>1607</td>
<td></td>
</tr>
<tr>
<td>Uracil</td>
<td>371</td>
<td>511</td>
<td>515</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

* IBR infected MDBK cells were pulsed for various periods of time with 5-3H-uridine. The virus DNA was purified by centrifugation to equilibrium twice in CsCl. The virus DNA was hydrolysed and the bases were separated chromatographically. The distribution of radioactivity was determined by cutting 3 mm strips and counting in a scintillation counter. One ml samples of pure 5-3H-cytosine and 5-3H-uridine (Amersham Searle) were treated in the same way to determine the relative distance of migration of each individual base.
† Radioactivity (ct/min), above background (39 ct/min), migrating to the position of labelled cytosine or uridine standards treated in a similar way.

the virus DNA. To test this possibility, extracted DNA was analysed with respect to its susceptibility to RNase and DNase or hot alkali. Table 1 indicates that IBR nucleic acid labelled for short periods (20 min) was highly susceptible to RNase and alkali but not to DNase. However, increasing the labelling time resulted in a gradual increase in susceptibility to DNase and a decrease in susceptibility to RNase and alkali. These results suggest that 5-3H-uridine can be incorporated directly into IBR nucleic acid and that 5-3H-uridine can also be converted to 5-3H-cytosine which can subsequently be incorporated into DNA.

A second approach used to determine if 5-3H-uridine was incorporated directly into the IBR viral nucleic acid in the form of RNA, was to hydrolyse the purified DNA and to determine by chromatography the distribution of radioactivity among the bases. Table 2 indicates that after a short pulse, with 5-3H-uridine, all of the label was recovered as uracil whereas if the labelling period was 2 h or longer, a considerable amount of radioactivity was associated with cytosine, indicating that conversion from 5-3H-uridine to 5-3H-cytidine did occur. However, this conversion to cytidine was not complete since DNA extracted from mature viral nucleocapsids, after 20 h of labelling, still contained some uracil.
Fig. 5. Effect of heat on the buoyant density of $^{3}$H-uridine labelled IBR DNA purified by two CsCl equilibrium gradient centrifugations. (a) IBR DNA labelled with $^{3}$H-uridine ($5 \times 10^4$ ct/min) centrifuged to equilibrium in Cs$_2$SO$_4$. (b) Purified IBR DNA (< 1 µg/ml) labelled with $^{3}$H-uridine ($5 \times 10^4$ ct/min) in 0.1 x SSC at 100 °C for 10 min, rapidly chilled on ice and centrifuged to equilibrium in Cs$_2$SO$_4$. Fractions were collected from the bottom of the tube.

**Linkage of $^{3}$H-uridine labelled RNA to IBR virus DNA**

To establish if the $^{3}$H-uridine was linked to IBR viral DNA through hydrogen or covalent bonds, the purified virus DNA labelled for 30 min with $^{3}$H-uridine was heated at 100 °C for 10 min. The denatured DNA was then rapidly cooled on ice before centrifuging to equilibrium in Cs$_2$SO$_4$. Fig. 5(a) illustrates that native IBR DNA sediments at a density characteristic of double stranded DNA. Denaturation by heat as illustrated in Fig. 5(b), resulted in a shift of the radioactivity to the density characteristic of single stranded DNA and not to the density of RNA. If the $^{3}$H-uridine was in the form of RNA attached to the DNA by hydrogen bonds it should have separated from the DNA during heating and would band at the density of RNA (1.60 to 1.65 g/ml) in a Cs$_2$SO$_4$ gradient. This did not occur, therefore, the RNA is probably covalently linked to the viral DNA.

**DISCUSSION**

Newly synthesized DNA of herpes simplex virus and certain bacteriophages has recently been shown to contain regions of RNA-like material (Speyer, Chao & Chao, 1972; Magnusson *et al.* 1973; Rosenkranz, 1973; Biswal *et al.* 1974; Murray & Biswal, 1974). The function of this RNA remains ill-defined but it has been suggested that the RNA may serve as a primer for DNA synthesis (Brutlag, Scheckman & Kornberg, 1971; Sugino *et al.* 1972;
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Wickner et al. 1972; Pato, 1975). Our results serve to indicate that RNA also occurs in the genome of IBR virus a bovine herpes virus.

We showed that a considerable amount of 5-3H-uridine label sedimented with IBR virus nucleocapsids and that virus DNA extracted from these nucleocapsids also contained radioactivity. Several possible explanations could be given to account for the presence of label sedimenting with the viral nucleocapsids. (1) Contaminating cellular RNA attached to the surface of the viral nucleocapsids. (2) Small pieces of cellular or viral RNA accidentally encapsulated into the virion during assembly. (3) Viral or cellular RNA present as an internal component associated with the internal proteins or attached to the DNA by a protein bridge. (4) 5-3H-uridine is converted to cytidine and subsequently incorporated into virus DNA. (5) The label is in the form of RNA covalently linked to IBR DNA.

Possibility (1) could be excluded by showing that treatment of the nucleocapsids with RNase prior to sedimentation did not alter the amount of radioactivity associated with the nucleocapsids nor did it alter the sedimentation rate of the label. These results suggest that the label was not due to RNA adhering to the surface of viral nucleocapsids. The second possibility was excluded by showing that purified viral DNA extracted from methyl-3H-thymidine or 5-3H-uridine labelled nucleocapsids sedimented at the same rate. Such a result would not be expected if the label was not part of the viral nucleic acid. Deproteinization of this labelled nucleic acid had no effect on the density of the labelled nucleic acid suggesting that the RNA was not bound to the virus DNA by a protein.

The fourth explanation for our results could be that 5-3H-uridine could be converted to deoxycytidine and subsequently incorporated into viral DNA (Rosenkranz, 1973). This possibility was shown to be partially correct in that when purified viral DNA, isolated from nucleocapsids, was hydrolysed into individual nucleotides and separated chromatographically, the majority of the label was in the form of cytosine. However, in all experiments conducted, there was always a small but significant percentage of the label present as uracil (Table 2). This uracil was probably in the form of RNA covalently linked to the viral DNA since the buoyant density of the label did not shift to the region of RNA following heat denaturation and centrifugation to equilibrium in Cs2SO4.

The function of this RNA in the viral genome is not definitely known at present, however, it has been suggested that it may act as a primer for DNA synthesis (Biswal et al. 1974). If this is the case then one would expect newly synthesized IBR DNA to contain small pieces of RNA. Once DNA replication was proceeding this RNA would then be removed and therefore mature DNA should contain no or less RNA. Our results support this hypothesis in that viral nucleic acid (DNA) isolated after pulsing for short periods (20 min) with 5-3H-uridine, contained large amounts of RNase sensitive material whereas DNA isolated from mature nucleocapsids contained considerably less RNase sensitive material, possibly due to the excision of the RNA after DNA synthesis was completed. In addition to serving as a primer, the RNA, which is retained after synthesis is complete, may have other specific functions and may occupy unique positions in the viral genome. A third possibility is that the presence of RNA in mature viral DNA may be due to an erroneous insertion of ribonucleotides during DNA polymerization (Blair et al. 1972). If this is the case, one would expect the RNA to occur at random positions. Our present results cannot distinguish between these possibilities.

Various models for the structure of HSV-1 DNA have been proposed. Frenkel & Roizman (1972) favour the model wherein alkaline labile regions occur in unique positions in only one of the strands. Wilkie (1973) presents evidence to support a second model wherein the alkaline labile regions occur in both strands of the duplex. Experiments are presently in
progress to determine whether alkaline labile regions are always associated with ribonucleotides and whether these occur in both strands of IBR DNA, their location and possibly the size of these ribonucleotide regions.

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REFERENCES


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