HOST-CELL MACROMOLECULAR SYNTHESIS AND VIRAL RNA SYNTHESIS IN RINDERPEST VIRUS INFECTED CELLS

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ABSTRACT

The syntheses of cellular DNA, RNA and protein were studied in Vero cells infected with Rinderpest virus. The DNA synthesis was inhibited to a significant extent starting from the early period upto the late period after virus infection. The effect on RNA synthesis was negligible in the early period, but it was inhibited in the late period after virus infection. No significant change in protein synthesis was observed during the early and late periods after virus infection. The synthesis of viral RNA was then studied in infected cells after inhibition of cellular RNA synthesis by using Actinomycin D and it was observed that viral RNA synthesis is completed in 48 hours after infection.

1. INTRODUCTION

RINDERPEST virus (RPV) is a negative stranded RNA virus belonging to the paramyxo group of animal viruses and is antigenically related to measles and canine distemper virus. Studies on the effect of New Castle disease virus (NDV), a member of paramyxo group, on cellular macromolecular synthesis have shown significant inhibition of DNA, RNA and protein syntheses after virus infection[1,2]. On the other hand, studies on SV5 virus, which also belongs to the same group, showed no significant inhibition of cellular DNA, RNA and protein syntheses after virus infection[3].

In the light of the above reports, we have made an attempt to study the host-cell-virus interaction by following the syntheses of cellular DNA, RNA and protein in Vero cells infected with Rinderpest virus. Studies with specific reference to viral RNA synthesis have also been made by shutting off cellular RNA synthesis to an extent of 95% in infected cells by using Actinomycin D. In addition, the different types of RNAs produced in infected cells have also been studied by employing rate zonal centrifugation.

2. MATERIALS AND METHODS

The radioactive isotopes ^{3}H-thymidine (Sp. Activity 10500 mCi/m mol), ^{3}H-Uridine (Sp. Activity 9700 mCi/m mol) and ^{14}C-algal protein hydrolysate (Sp. Activity 42 mCi/milli atom C) used in the study were obtained from the Bhabha Atomic Research Centre, Bombay. Eagle's Minimum Essential Medium (MEM) was obtained from Centron Research Laboratories, Bombay. Actinomycin D and density gradient grade sucrose were obtained from Sigma Chemical Co., USA. All other chemicals used were of analytical grade.

2.1 *Cells and virus used.*—Vero cells, a continuous cell line derived from African green monkey kidney, were used for the propagation of the virus. Rinderpest virus, Kabete 'O' Vaccine strain (obtained from Serum Institute, Bangalore) was used in the present study. Vero cells were grown using 'Eagle's minimum essential medium fortified with non-essential aminoacids, 4% goat serum and antibiotics in 10 ml screw capped tubes at a cell density of $0{\cdot}4 \times 10^{6}$ cells/ml medium. Rinderpest virus, having a titre of 10^{4} TCID50 was used. Multiplicity of infection was 0·25. Virus adsorption was done at 37° C for 1 hour. Uninfected cells of the same batch were put up as controls.

2.2 *Incorporation studies.*—Incorporation studies were carried out at 24 hour intervals after virus infection by incubating the monolayers in 1 ml of medium at 37° C for 4 hours in the presence of 10 μCi/ml each of ^{3}H-thymidine and ^{3}H-uridine and 1 μCi of ^{14}C-Algal protein hydrolysate. Hank's balanced salt solution fortified with 4% goat serum was used as culture medium when protein synthesis was studied.

Infected cells were pre-treated with Actinomycin D (10 μg/ml) for 2 hours at 37° C before addition of 10 μCi/ml of ^{3}H uridine (Sp. Act. 9700 mCi/m mol) when viral RNA synthesis was being followed[4].

After incubation, the cells were washed with phosphate buffered saline and mixed with 0·5 ml of tris-magnesium buffer, pH 7·4, containing 0·1% sodium dodecyl sulphate. Equal volumes of cell-lysate were used to determine cold trichloracetic acid insoluble radioactivity. Radioactivity was determined in a Beckman LS 100 liquid scintillation spectrometer.

2.3 *Extraction of viral RNA and characterisation.*—RNA was extracted from infected Vero cells pre-treated with Actinomycin D for 2 hours and then incubated for 8 hours in the presence of 10 μCi/ml ^{3}H uridine (9700 mCi/m mol) at 37° C. The pre-treatment and incubation with label were done 24 hours post-infecion.

Extraction was done by using SDS–Phenol and precipitating with 2 vols. of ethanol after addition of yeast carrier RNA (500 μg/ml). The precipitated RNA was washed with ethanol : STE (sodium chloride-Tris-EDTA buffer, pH 7·4) and then layered on 15–30% sucrose (w/w in STE) linear gradients and centrifuged in a SW50 rotor in Beckman L2 ultracentrifuge at 45000 rpm for 4 hours at 4° C. Vero Marker RNA was included in the run.

3. Results and Discussion

The inhibition of cellular DNA synthesis after virus infection is shown in Fig. 1. Cellular DNA synthesis was inhibited to a significant extent, the final percentage of inhibition being as high as 90. It is probable that Rinderpest virus codes for a protein which inhibits cellular DNA synthesis or perhaps inhibits it, *via* its effect on the host DNA polymerase. Inhibition of cellular RNA synthesis occurs in the late period after infection only.

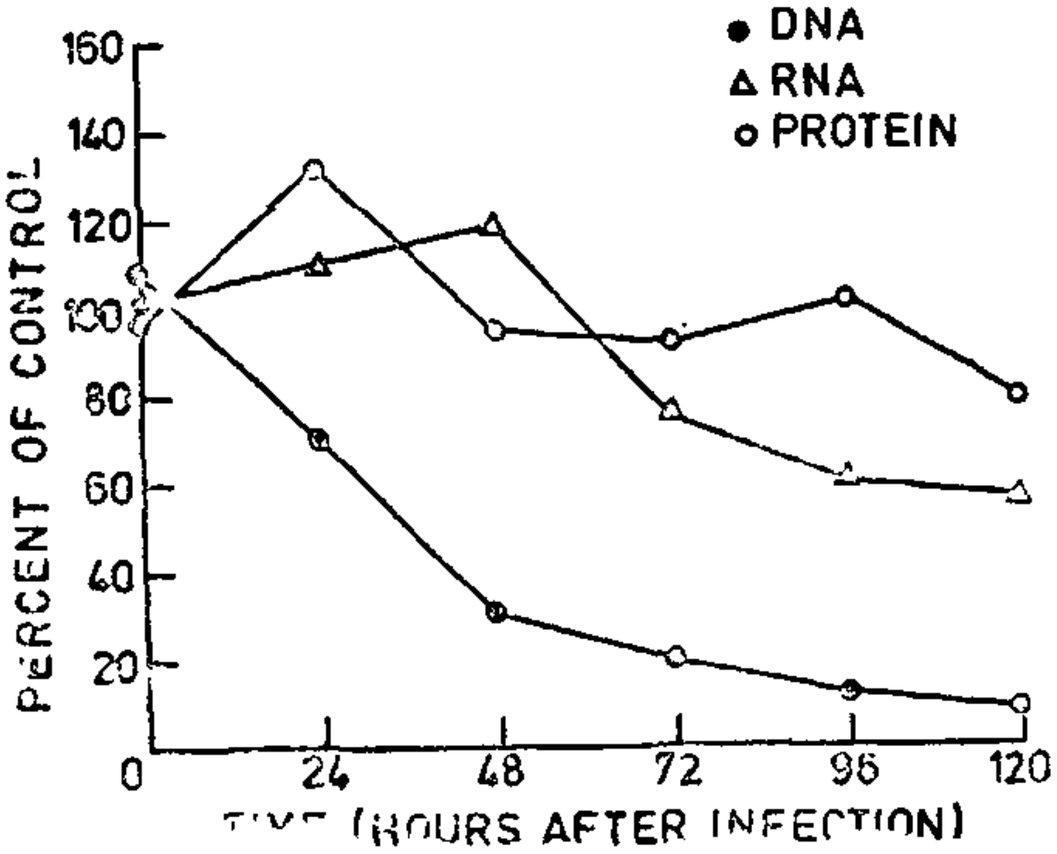

Fig. 1. Profiles of cellular DNA, RNA and protein syntheses after infection with Rinderpest virus.

This may probably be a result of marked cytopathic effect (CPE) which occurs only in the late period of infection when the infected cells get rounded up, begin to coalesce and disturb the cellular integrity. The effect of RPV infection on the cellular protein synthesis is negligible. Studies on Reovirus, a double-stranded RNA virus, have shown that there is a specific inhibition of cellular DNA synthesis without concurrent inhibition of cellular protein synthesis[5,6].

The viral RNA synthesis profile is shown in Fig. 2. Actinomycin D, a potent inhibitor of DNA dependent RNA synthesis, was used at a concentration of 10 μg/ml which inhibited host RNA synthesis to an extent of 95%. The data obtained show that viral RNA synthesis is completed by 48 hours and thereafter it declines. It appears that the synthesis of viral mRNAs (+ strand species) and genomic RNA is completed by 48 hours.

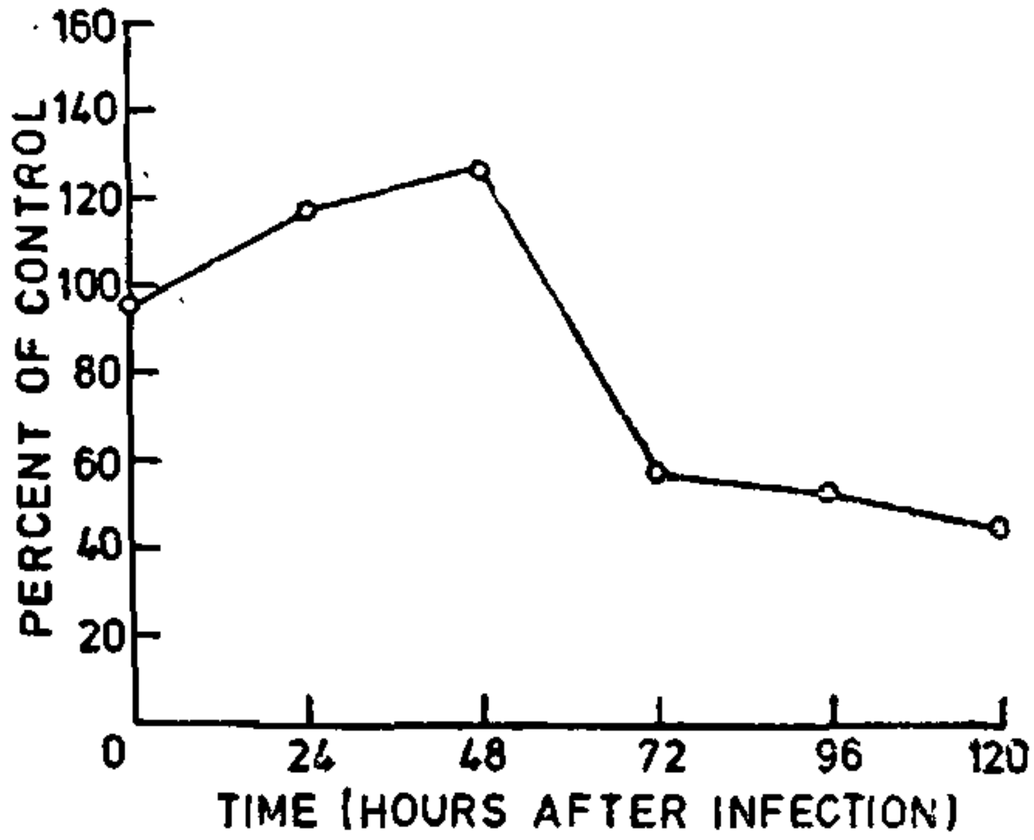

Fig. 2. Viral RNA synthesis in infected Vero cells treated with Actinomycin D (10 μg/ml) to shut off cellular RNA synthesis.

Velocity sedimentation analysis of viral RNA on sucrose linear gradients have shown that three species of viral RNAs are produced (Fig. 3) in

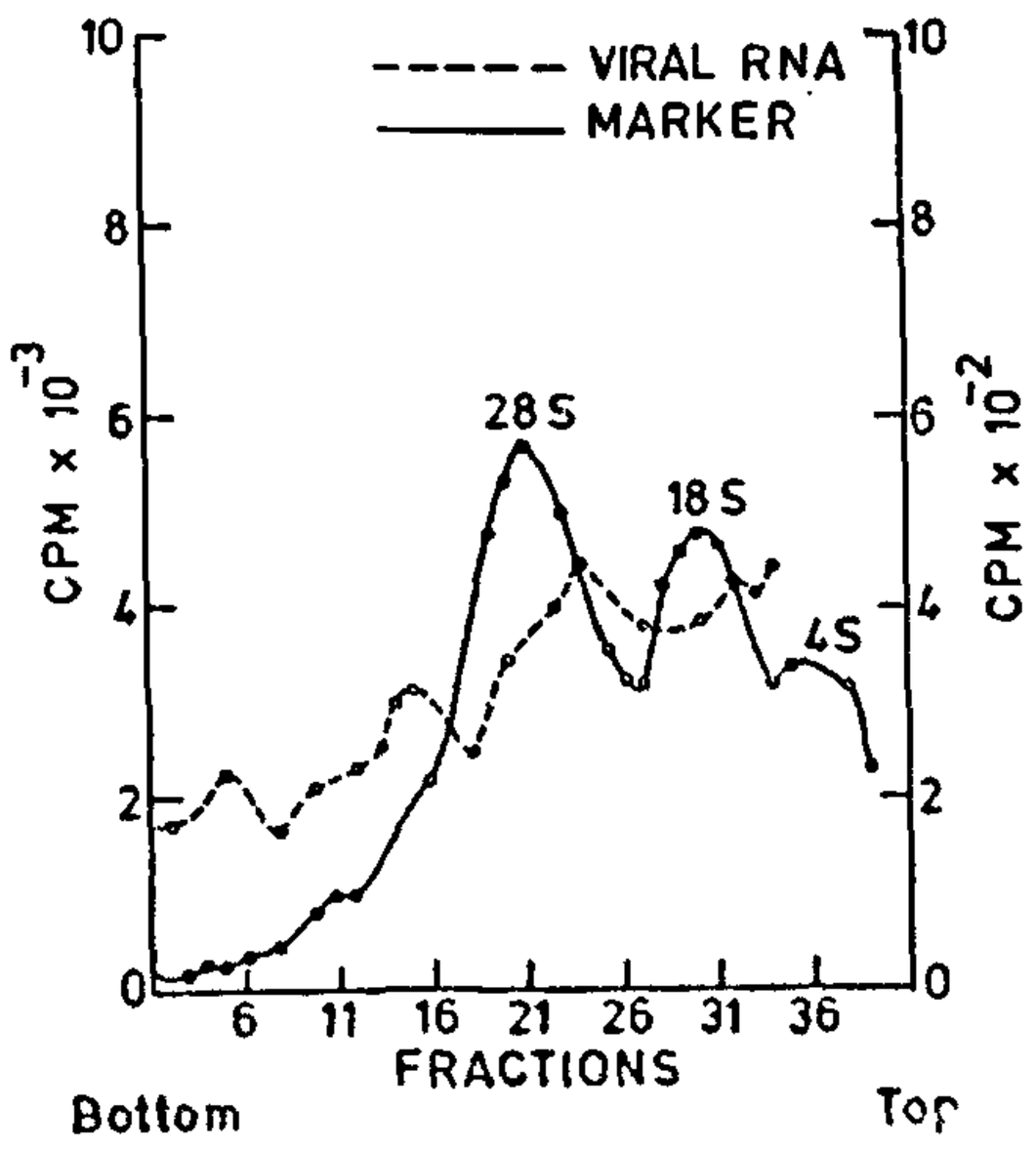

Fig. 3. Velocity sedimentation analysis of viral RNA run on 15–30% sucrose (w/w in STE) linear gradients. Centrifugation was done in a SW50 rotor at 45000 rpm for 4 hours at 4° C. The three peaks have been assigned 50–55S, 35S and 18–22S values as seen from bottom to top.

infected cells. By comparison with the S values of marker RNA, the three species have been assigned 50–55S, 35S and 18–22S values as seen from bottom to top. This is in agreement with the results obtained with other paramyxoviruses like New Castle disease virus[7,8] SV5[9] and Sendai[10].

The interesting fact to be noted is that even at a very low m.o.i. (0·25), there is drastic inhibition of DNA synthesis and RNA synthesis is also inhibited in the early stages of infection. Thus, our results indicate that during RPV infection cellular DNA and RNA syntheses are inhibited, the former to a greater extent whereas cellular protein synthesis is almost unaffected. Efforts are under way to characterise further the viral RNA species and virion RNA by fingerprint analysis.

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The Central Leather Research Institute will be celebrating its Silver Jubilee in 1978 and the Jubilee activities will be inaugurated on 31st January 1978. The activities include the 'Leather Week' from Jan. 31st to Feb. 6th covering a Seminar on ('Leather Auxiliaries" (Feb. 1–3), and International "Leather Fair (31st Jan.–6th Feb.) followed by a large number of other activities (International Seminars on "Leather Goods", "Transfer of Technology and Scientific and Industrial Information Services", lectures, group discussions, activities promoting exchange of knowledge, information and experience, etc.) throughout the year.