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THE NUCLEIC ACID OF JUNÍN VIRUS

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Sumary. — The replication of Junín virus in BHK cells was not inhibited by 5-bromodeoxyuridine (5-BUDR) in a 15 μg/ml concentration. Actinomycin D did not inhibit viral replication during the first 48 hours of viral growth, but in a concentration as low as 0.05 μg/ml it reduced the virus yield after 48 hours of treatment. The results indicate that neither DNA synthesis nor DNA-dependent RNA synthesis is required for virus synthesis, and should be interpreted as indirect proof that Junín virus is of RNA type.

Introduction

Junín virus, the aetiological agent of Argentine Haemorrhagic Fever, has been classified as an arbovirus, member of the Tacaribe group, although its nucleic acid type has not been definitely established. The low yield of virus from infected tissues and cells and the fact that the virus has not yet been concentrated and purified to a degree allowing chemical determination of its components (Martínes Segovia and Díaz, 1968) is a hindrance for this purpose.

The role of DNA was studied in BHK cell cultures using inhibitors with a known effect on the synthesis or function of DNA. The metabolic analogue 5-BUDR and the antibiotic Actinomycin D, which inhibit the multiplication of DNA viruses, were employed in experiments designed to obtain indirect evidence of the possible role of DNA in viral synthesis. As is known, 5-BUDR, a thymidine analogue, can be incorporated into cellular DNA in place of thymine, and Actinomycin D combines with guanine DNA, thus inhibiting DNA-dependent RNA synthesis.

Susceptibility of BHK cells to Junín virus has been previously reported (Martínez Segovia *et al.*, 1967).

Materials and Methods

Viruses. The following viruses were employed: 1) Junín virus, strain MC_2, 12th intracerebral mouse passage; 2) Aura virus, strain A Serratus obtained from Dr. Barrera Oro (Barrera Oro *et al.*, 1967) in the 7th mouse brain passage; 3) herpes simplex virus, after 4 passages in rabbit kidney cells, 7 passages in chorioallantoic membranes and one passage in BHK cells.

Stock viruses. Junín and Aura viruses were propagated by intracerebral inoculation of newborn mice. Brains from infected mice were removed 7 days after inoculation for Junín virus, and at the time the animals were moribund for Aura virus. Twenty per cent suspensions were prepared in Hanks' balanced salt solution containing 10% foetal bovine serum. The suspensions were centrifuged at 700 × g for 15 minutes and the supernatants kept frozen at —70° C as virus stocks. Herpes-infected fluids from BHK cell cultures were harvested when the latter showed advanced cellular degeneration. After centrifugation at 700 × g for 15 minutes, the supernatants were stored as above.

Cell cultures. BHK 21 cells were employed to prepare the virus stocks and for virus titrations. The cells were grown in Eagle's medium suplemented with 10% of tryptose phosphate broth and 10% of foetal bovine serum. Maintenance medium (MM) contained only 2% of heated foetal bovine serum.

Virus assay. Infectivity of virus from culture fluids and cells was determined as follows: Aura and Junín viruses were titrated by intracerebral inoculation of tenfold dilutions into suckling mice. The LD_{50} values were calculated by the Reed and Muench method. Herpes simplex virus was titrated in BHK cell monolayers. Sets of 4 tubes were each inoculated with one ml of serial 10-fold dilutions of virus in MM. Readings were made when the cytopathic effect (CPE) was complete in control tubes. Titres were recorded as the reciprocal of the dilution causing 50% CPE.

Results

Toxicity of 5-BUDR

Preliminary experiments were carried out to determine the highest amount of 5-BUDR tolerated by BHK cells. Morphological appearance and cell numbers were used as indicators. At a concentration of 30 μg/ml, 5-BUDR

Table 1. Effect of 5-BUDR (15 μg/ml) on the multiplication of BHK cells

Days after addition of 5-BUDR	Number of cells (millions)		Decrease in number of cells
	Without 5-BUDR	With 5-BUDR	
1	20.8	15.2	1.35×
2	22.4	17.0	1.31×
4	21.6	9.5	2.27×
5	20.0	9.0	2.22×
7	19.0	8.5	2.23×
9	17.5	7.5	2.33×

provoked a marked decrease in the number of cells, and a progressive cytotoxicity judged by visible morphological alteration. At a concentration of 15 μg/ml, the number of cells decreased slightly (1.3-fold) after 24 hours,

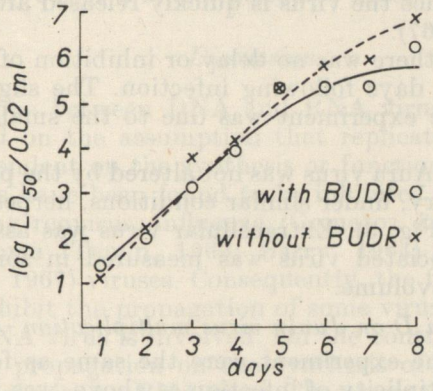

Fig. 1.

Effect of 5-BUDR on the growth of Junín virus in BHK cell cultures
Abscissa: days after infection

and a 2.3-fold decrease accompanied by cell rounding was seen throughout the monolayer at the 4th day (Table 1). The latter concentration was then used throughout.

Effects of 5-BUDR on growth of Junín virus and control viruses

The cells were simultaneously exposed to Junín virus and the drug. The multiplicity of infection was 1.2. For control purposes an RNA virus (Aura; Z. Martínez Segovia, unpublished data) and a DNA virus (herpes simplex)

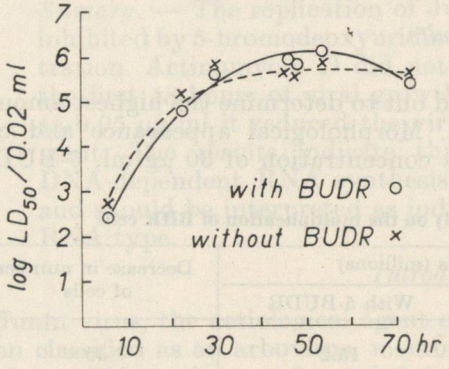

Fig. 2.

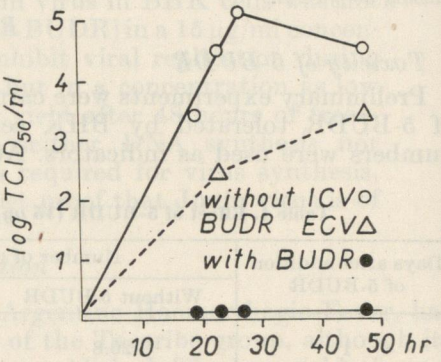

Fig. 3.

Fig. 2.

Effect of 5-BUDR on the growth of Aura virus in BHK cell cultures
Abscissa: hours after infection

Fig. 3.

Effect of 5-BUDR on the growth of herpes simplex
ICV and ECV: intra- and extra-cellular virus, respectively
Abscissa: hours after infection

were included into each experiment. We determined the titres of Junín virus in the culture fluids since the virus is quickly released after formation (Martínez Segovia *et al.*, 1967).

As shown in Fig. 1, there was no delay or inhibition of Junín virus multiplication during the 6 days following infection. The slight decline of virus titre at the end of the experiment was due to the smaller number of cells present at that time.

The growth curve of Aura virus was not altered by the presence of 5-BUDR (Fig. 2). On the contrary, under similar conditions, herpes simplex virus was completely inhibited (Fig. 3). Extracellular virus was assayed in the supernatant, while cell-associated virus was measured in sonicated cells resuspended in the original volume.

Effect of Actinomycin D on Junín virus multiplication

The conditions of the experiment were the same as for 5-BUDR. Junín virus, at the same multiplicity of infection as above, was inoculated simultaneously with 0.5 and 0.05 μg/ml of Actinomycin D on BHK cell cultures. At intervals, the medium was harvested and the virus yields were determined.

Table 2. Effects of Actinomycin D on the growth of Junín, Aura, and herpes simplex viruses

Viruses	Hours after infection	Infectivity titres (log values)			
		0.5 μg/ml of Actinomycin D		0.05 μg/ml of Actinomycin D	
		control	treated	control	treated
Junín	24	0.5	1.0	0	0.5
	48	1.1	1.5	2.2	2.0
	77	3.0	1.7	ND	ND
	144	ND	ND	4.8	3.8
Aura	24	4.7	4.3	5.2	5.6
	48	5.6	5.3	5.8	5.8
Herpes simplex	24	3.5	0	3.5	0
	48	4.5	0	4.5	0

0 = no virus detected. ND = not done. Actinomycin D was added at time 0.

As shown in Table 2, there was no inhibition of virus multiplication during the first 48 hours after the addition of the antibiotic. At 77 hours or later, 0.5 μg/ml of the drug moderately reduced the virus yield. The long growth cycle of Junín virus may be a factor in the decline of virus yield, which occurs presumably through deleterious effects on the host cell. The results of two representative experiments on control viruses included in Table 2 show that the multiplication of Aura virus was not impaired in the presence of Actinomycin D, whereas herpes simplex virus was completely inhibited. With a smaller concentration of the antibiotic (0.05 μg/ml), the cells were less affected, herpes simplex virus was still completely inhibited, the growth of Junín and Aura viruses was not influenced and the multiplication of Junín virus could be followed for a longer period of time.

Discussion

The differentiation between DNA and RNA viruses by the use of DNA inhibitors is based on the assumption that replication of RNA-containing viruses is not dependent on the synthesis or functioning of DNA. However, some RNA viruses have been found to be inhibited by 5-BUDR or Actinomycin D, including reovirus, influenza (Gomatos *et al.*, 1962; Barry *et al.*, 1962), Rous sarcoma (Temin, 1963; Barry, 1964) and Murine leukaemia (Bases and King, 1967) viruses. Consequently, the finding that 5-BUDR or Actinomycin D inhibit the propagation of some viruses, does not necessarily indicate that a DNA virus is involved. On the contrary, the absolute independence of virus propagation on the synthesis of cellular DNA indicates that we dave to deal with an RNA virus. The present results with Junín virus indicate that the replicative mechanism is insensitive to inhibition by 5-BUDR and Actinomycin D. The multiplication of this virus can, however,

be reduced by the Actinomycin D under certain circumstances, pressumably through deleterious effects on the host cells.

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