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The nucleic acid of African horse sickness virus

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INTRODUCTION

The arboviruses are accepted as a group of RNA-containing viruses which are sensitive to ether and which multiply in and are transmitted by arthropod vectors. African horse sickness virus (AHS virus) is usually classified in this group (Spradbrow, 1964) although its nucleic acid type has not yet been established.

This paper presents some studies on the nature of AHS virus nucleic acid. The effects of actinomycin D on virus growth were examined. This compound combines selectively with DNA, and inhibits DNA-dependent RNA synthesis (Reich, Franklin, Shatkin & Tatum, 1962; Goldberg, Rabinowitz & Reich, 1962).

MATERIALS AND METHODS

Virus

AHS virus mouse-adapted type 2 (OD) and type 6 (S. 114) were used. The stock AHS virus was a pool of 5th to 10th passage virus in MS cells, lyophilized and stored at $-40°$ C. The titres of the AHS viruses were 2 and 6×10^7/ml. plaque-forming units (pfu).

Cells

Line MS of monkey kidney cells was grown in tubes or in 2 oz. bottles for 3–4 days. The growth medium consisted of 5% lactalbumin hydrolysate in Earle's balanced salt solution with the addition of 0·005% yeast extract, 5% inactivated calf serum, and antibiotics. The maintenance medium was the same, but contained only 2·5% calf serum. Adaptation of AHS virus to MS cells has been described in previous reports (Mirchamsy & Taslimi, 1964*b*, 1964*c*).

Drug

Actinomycin D (AD) was kindly supplied by Dr B. Roizman of the Department of Epidemiology, Johns Hopkins University, Baltimore, U.S.A. A stock solution was prepared in sterile 0·85% NaCl at a concentration of 200 μg./ml. This solution was stored in the dark at 0° C. Further dilutions were made in the maintenance medium just before use.

Plaque assay

AHS virus was titrated by a plaque assay method, previously described in detail (Mirchamsy & Taslimi, 1966).

RESULTS

Effect of actinomycin D on the growth of MS cells

Concentrations of 5 μg./ml. or more of actinomycin were toxic for MS cells. The morphological changes were most marked by the second day, when cells had stopped growing. By 24 hr. a noticeable increase in cytoplasmic volume was observed.

The effects of smaller concentrations of actinomycin on cell growth were investigated by counting the number of viable cells in treated and untreated cultures daily for 5 days. The results of a representative experiment are listed in Table 1. Daily observation of actinomycin treated cells indicated that with concentrations up to 0·5 μg./ml. only small foci of cells were granulated. This change became more marked with higher doses, until severe nuclear and cytoplasmic damage or complete cell lysis was observed three days after addition of 2·5–5 μg./ml. of the drug.

Table 1. *Effect of actinomycin D on the growth of MS cells**

Concentration of AD (μg/ml)	Per cent surviving cells after:				
	1 day	2 days	3 days	4 days	5 days
0	95	90	84	81	79
0·05	90	88	80	75	69
0·075	88	85	80	73	68
0·1	85	80	74	70	64
0·25	80	76	73	69	60
0·5	76	71	67	61	54

* Cells from two tubes for each dose of actinomycin D were examined daily. The cells were treated with trypsin, harvested, and then stained with 0·1 % trypan blue.

Effect of actinomycin D on free virus

A suspension of AHS virus type 6 containing 200 pfu/ml. was mixed with 1 ml. of Dulbecco's phosphate buffer saline containing 0·3 μg./ml. of actinomycin, and the mixture was incubated 2 hr. at 36° C. A control sample without drug was included. The samples were then assayed to determine the amount of surviving virus. No change was found under the above treatment.

Virus growth curves

In these experiments, batches of MS cell cultures were infected with AHS virus type 2 or 6 at an approximate multiplicity of 2. After 90 min. at 36° C. to allow adsorption the cultures were washed twice in phosphate buffered saline and re-incubated in 1 ml. of maintenance medium. At 6-hourly intervals pairs of cultures were disrupted by five cycles of freezing and thawing. After centrifugation for 5 min. at 2000 r.p.m. the supernatants were assayed for virus content by the plaque assay method. To determine the effect of actinomycin, batches of MS cells were pre-incubated for 8 hr. with actinomycin D at concentrations of 0·1, 0·25 and 0·5 μg./ml. before addition of virus. The results of a typical experiment are shown in Fig. 1.

In control cultures, AHS virus at a multiplicity of two particles per cell has an eclipse period of 6–8 hr. In cultures treated with 0·1 or 0·25 μg./ml. of actinomycin the eclipse period is much longer, being 12 and 24 hr., respectively, and the final virus yields are much lower than those of the controls. Cultures treated with 0·5 μg./ml. of actinomycin do not seem to produce significant amounts of AHS virus. The inhibition of virus growth by these doses of actinomycin during the first 24 hr. after infection seems to be selective, as no morphological change or significant loss of cell numbers has been observed.

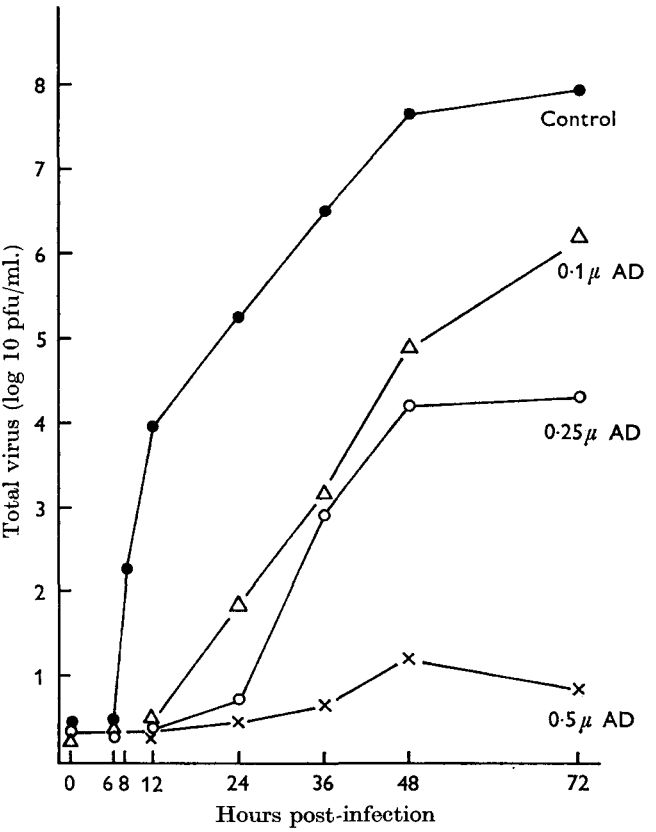

Fig. 1. Growth of African horse sickness virus type 6, strain 114 in MS cells infected at a multiplicity of 2. Cells pretreated with: △, actinomycin D 0·1 μg./ml.; ○, AD 0·25 μg./ml.; ×, AD 0·5 μg./ml.; ●, untreated control.

DISCUSSION

African horse sickness virus is regarded as an arbovirus because of its host range, tissue tropism and the need for vector transmission (Andrewes, 1962). Cooper (1961) has proposed a system of classification based on type of nucleic acid and sensitivity to ether. All arboviruses so far isolated have been found sensitive to ether (Casals, 1961) and to sodium deoxycholate (Theiler, 1957). However, AHS is insensitive to these chemicals (Howell, 1962). Furthermore, there is some doubt whether AHS virus actually multiplies in the insect vector or is

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merely transferred mechanically (Ozawa & Nakata, 1965; Mirchamsy & Taslimi, unpublished).

If AHS is not an arbovirus, more information about its chemical make-up is required before it can be classified. Attempts to isolate an infectious nucleic acid by several improved methods of phenol extraction (Wecker, 1959; Di Mayorca *et al.* 1959) have failed (Mirchamsy, Taslimi and Kamali, unpublished data). Consequently, the susceptibility of virus multiplication to actinomycin D was tested, as an indirect means of establishing whether virus nucleic acid synthesis was of the RNA or DNA type.

AHS virus multiplication is inhibited by actinomycin D. This could mean that it is a DNA-containing virus. There are, however, a number of RNA-containing viruses that are also inhibited—influenza virus (Barry, Ives & Cruickshank, 1962), Rous sarcoma virus (Temin, 1963) and visna virus (Thormar, 1965). At least in the case of influenza virus it has been suggested (Barry *et al.* 1962) that virus RNA is made on a DNA template, and that blockage of the template by actinomycin inhibits virus production. On the other hand, the antibiotic may have some effect on the general metabolism of cells which affects some early stage in the growth of susceptible viruses (Temin, 1963). Fluorescent antibody studies (Mirchamsy & Taslimi, 1964*a*) and acridine orange studies (Mirchamsy & Taslimi, unpublished observations) suggest that, like influenza virus, AHS virus multiplies in the nucleus of the infected cell. Since the growth of AHS is sensitive to low concentrations of actinomycin, it is likely that this virus either contains DNA as its genetic material or that there is a stage in virus replication that is dependent on cellular DNA.

SUMMARY

The effect of actinomycin D on the growth of African horse sickness virus in monkey kidney cells was studied. It was found that actinomycin D inhibited the yield of virus. It is suggested that African horse sickness virus is either a DNA containing virus or an RNA virus whose replication is DNA-dependent.

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