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KINETICS OF RNA SYNTHESIS FOR CYTOPATHOGENIC AND NON CYTOPATHOGENIC BOVINE VIRAL DIARRHEA IRUS STRAINS

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INTRODUCTION

Bovine viral diarrhea virus (BVDV) is a member of the Togaviridae belonging to the genus pestivirus. Hafez and Liess (1972) suggested that BVDV had a single-stranded RNA (ss - RNA). Togavirus RNA has been shown to be a plus (+) strand, serving as a messenger RNA both in vivo (Simmons & Strauss, (1974; Mowshwitz, 1973; Soderlund et al., 1973) & Wengler and Wengler (1973) and in vitro (Smith et al., (1974; Simmons & Strauss, (1974). The naked RNA is infectious (Wecker, 1959). Diderholm and Dinter (1966) isolated an infectious RNA sensitive to RNase treatment. Pritchett et al., (1974), extracted RNA from isopycnic banded (H) - uridine labelled BVDV and showed that BVDV contained one major and two minor RNA species. The major RNA component was estimated to have a 38S sedimentation Co-efficient, whereas the minor RNA components were estimated at 31S and 24S. Analysis of the RNA molecules obtained from two strains, one cytopathogenic and one non-cytopathogenic, revealed that the 38-39S RNA was the major species of RNA in both strains, but different minor components were observed. Kaarianen and Gomatos (1969) found that togaviral RNA synthesis is easily observed in infected cells treated with actinomycin-D and labelled with a radioactive nucleic acid precursor such as ^{3}(H) uridine. Stollar et al. (1967) and Boulton and Westaway, (1977) observed that a pulse for 2 hours with a non-toxic dose of actinomycin-D (4 Ug/ml) was sufficient to reduce ^{3}(H) - uridine incorporation to 3-5% in unafrected cells compared to about 17% in Flavivirus infected cells in 24 hours. Attempts to enhance this effect are fraught with the hazard of induction of drug mediated cytotoxicity. No inhibition of togarvirus replication has been noted in the presence of actinomycin -D (Maes et al., 1967,

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Woods and Robbins, 1968, Wong et al., 1969; Hovi and vaheri, 1970; and Sedwick and Sokol, Aynaud, 1968, Horzinek, 1976 and Nuttall et al., 1978). Labelling with radioactive ^{3}(H) - uridine has been successful with most animal non-arbo Togaviruses; however, difficulties in labelling the RNA of pestiviruses have been reported by Nuttall (1978). The purpose of this study was to determine the kinetics of RNA synthesis between 4 cytopathogenic and fournoncyto-pathogenic BVDV strains and to examine the phenomenon of cytopathology in relation to nucleic acid production.

MATERIALS AND METHODS

Viral Strains:-

The cytopathogenic strains of bovine viral diarrhea virus (NADL, Oregon C V, Singer and Longnecker) ^{24} and noncytopathogenic strains (JS - 75, Draper, Morton and NY - 1). were used in this study.

Propagation of Viruses:-

All viruses were propagated in bovine turbinate cells (Btu). The Btu cells were grown in Eagle's minimal essential medium with Earle's salt, supplemented with 2% L - glutamine, 2% Sodium pyruvate, nonessential amino acids, 5ug/ml Amphotericin B, 50ug/ml gentamicin and 10% fetal calf serum. Cells were maintained in the same medium, except the serum concentration was reduced to 2% and fetal calf serum was replaced with horse serum to prevent BVDV - antibody in terference with viral replication. All Btu's used for viral replication were subjected to a thorough screening for adventitious BVDV using a specific fluorescent antibody test. Confluent Btu monolayers were washed with PBS and a viral inoculum was allowed to adsorb for two hours at 37°C after which maintenance medium was added to each culture. Infected and control cells were observed daily. For cytopathogenic BVDV strains, 70 - 90% CPE was usually observed within 3 - 4 days and for non-cytopathogenic strains bright green immuno - fluorescence was observed within 4 - 6 days in test cultures. When viral infections reached a maximum, the infected cells were frozen and stored at - 70°C. Before each experiment, viral strains were adjusted to similar multiplicities of infection.

Measurment of virion RNA synthesis:

Confluent monolayers of Btu cells were labelled with 2 uci/ml of ^{3}(H) - uridine (New England Nuclear). Labelling started after viral adsorption and continued for the entire length of the experiment. Different concentrations of actinomycin - D were used (5 ug/ml, 3 ug/ml and 2.5 ug/ml) depending on the time length of the experiments to avoid drugmediated cytotoxicity. Parellel tests for viral infectivity were run concurrently with the tests for virion RNA synthesis. (direct - FAT). The

incorporation of label into infected and control cells was stopped by washing the monolayers 4 times with ice-cold EBSS (Earle's Balance Salt Solution). Cells were ruptured by using 1% SDS in 0.01 M Na acetate (PH 5.0.). After lysing the cells, 80 ul samples were transfered onto Whatman 3MM filter papers and washed three times with 10% ice - cold trichloracetic acid (TCA), and then washed once for five minutes in ice-cold absolute alcohol. The filter papers were then dried overnight in an oven at 50°C and then counted in a Beckman LS 7000 liquid scintillation counter using a Toulene - BBOT scintillation cocktail.

Statistical Analysis:-

Analysis of variance (ANOVA), and the paired - test, were used to determine levels of significance for rates of RNA synthesis.

RESULTS

Five ug/ml of actinomycin - D inhibited host DNA - dependent - RNA synthesis. This dose was sufficient to reduce ^{3}(H) - uridine incorporation from 70% at 1 hour to 99% at 48 hours. (Data not shown). When eitht different strains of BVDV were tested for RNA synthesis, no significant levels of (H) - uridine incorporation were detected between hours 4 - 10 post infection. After (12 - 14 hours), however, the cytopathogenic BVDV strains (except NADL) showed significantly higher ^{3}(H) - uridine incorporation when compared to the non-cytopthogenic BVDV strains or when compared with non infected cell controls (Figure 1A and B). Cells infected with non-cytopatogenic BVDV strains displayed specific viral antigens when tested by fluorescent antibody tests, but significant levels of RNA synthesis were not detected over the time interval of the experiments (40 hourrs).

DISCUSSION

The data repoted for BVDV growth in cell cultures are widely divergent (Saurat et al., 1972) most probably because of lake of defined single - cycle conditions. After latent phase of 4 - 10 hours, the infectivity starts to increase exponentially as was reported by Saurat et al., (1972). This finding supports our observation for the kinetics of RNA synthesis for cytopathgenic BVDV strains. A latent phase was observed 4 - 12 hours post - infection before any increase in ^{3}(H) - uridine incorporation into viral RNA was noted.

The duration of the latent phase appears to depend upon the viral strain (Hafez and Liess, 1972) and the cell cullture system (Schiff and Storz, 1972), but is similar at various multiplicities of infection. Since only one type of cells was used (bovine turbinate), it was not possible to determine if cell differences affect the pattern of kinetics of RNA synthesis. In this study it was observed that the RNA synthesis reached maximum levels between 12 - 24 hours for the

cytopathogenic strains (peak at 12 - 16) and declines the reafter. This is supported by (Nuttall, 1978), who found that virus growth peaks at 12 - 24 hours post infection, upon which a plateau is reached.

Nuttall (1978) reported difficulty in obtaining ^{3}(H) - uridine labelled BVDV. Although the ^{3}(H) - uridine incorporation reported here for virion RNA was not very high, it was statistically significant when compared to controls using ANOVA and the paired t-test. All the cytopathogenic BVDV strains (Longnecker, Oregon C_{24} V and Singer with the exception of NADL) were compared by the ANOVA at 12 - 24 hours post-infection to deterimine whether differences existed. The paired t-test was used for the comparison between the individual viruses, revealing astatistically significant difference in acid-insoluble radioactivity in virus-infected cell cultures, (the values were as follows: Longnecker ($P-<0.01$) Oregon C_{24}V ($P-<0.048$) and Singer ($P-<0.01$) when compared to the cellcontrols for the incorporation of ^{3}(H) - uridine.

The cytopathogenic NADL stralin did not induce significant levels of ^{3}(H) - uridine incorporation into RNA when compared to cell controls. This might be attributed to the production of defective interfering practicles (DI) or it might be a contamination of NADL with a non-cytopathogenic BVDV strain. Guild and Stollar (1975) reported that cells infected at a high multiplicity of infection with Aro-togavirus stocks produced defective interfering particles. The (DI) particles Lacked intrinsic infectivity and both depended upon and interfered with the multiplication of standard virus. D1 particles can also affect viral RNA synthesis depending on the ration of D1 particles in an inoculum. If the ration of D1 particles to standard virus is large, then the total amount of viral RNA synthesis is decreased.

The results of this study showed that 3 out of 4 cytopathogenic BVDV strains synthesized nucleic acid at significantly higher levels compared to the non-cytopathogenic strains and this led to an hypothesis for BVDV strains in terms of role of RNA synthesis in affecting cytopathology. It has been proposed by several investigators that several lines of evidence point to the accumulation of RNA as a critical point in the determination of cytopanthic effects (Blair and Robinson, 1968). They suggested that differences in accumulation of 18S RNA might account for differences in cytopthogenic effects for Sendai virus and Newcastle disease virus (NDV) infections of chicken embryo cells. Preble and Youngner (1973) found that a variant of NDV obtained from persistently infected chicken embryo cells was devective in RNA synthesis. Reeve at al. (1970) suggested that the balance between synthesis and utilization of viral specific products in infected cells may be a determinant of cytopathology. This notion is reinforced by the non-cytopathogenic mutants which produce infectious virus in amounts similar to AV - WT (A variant of

NDV) even though they accumulate both less RNA and less protein. It is conceivable that cytopathogenic BVDV strains have less RNA or protein regulation or induce the synthesis of a signficantly greater amount of double stranded RNA as an intermediate for single stranded RNA when compared to non-cytopathogenic BVDV strains. This might explain differences in cytopathogenicity for BVDV strains.

SUMMARY

The kinetics of RNA synthesis between 4 cytopathogenic and 4 non-cytopathogenic bovine viral diarrhea virus (BVDV) strains were determined to examine the phenomenon of cytopathology in relation to nucleic acid production. All cytopathogenic BVDV strains except NADL showed significantly higher $(H)^3$ - uridine incorporation when compared to non-infected control cells. For the cytopathogenic strains. a latent phase was observed 10 hours post-infection before any increase in (H) -uridine incorporation into viral RNA was noted. Maximum levels of RNA synthesis occurred between 14 and 16 hours. When compared to control cells, no significant int incorporation of (^3H) - uridine was observed for the non-cytopathogenic BVDV strains. It is postulated that the difference between cytopathogenic and non-cytopathogenic BVDV strains may be due to replication events closely associated with and dependent upon virion RNA synthesis.

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Figure A
A composite comparing PNA synthesis for different cytopathogenic BVDV strains in Bth cells.

Figure B
A composite comparing RNA synthesis for different non-cytopathogenic BVDV strains in Btu cells.

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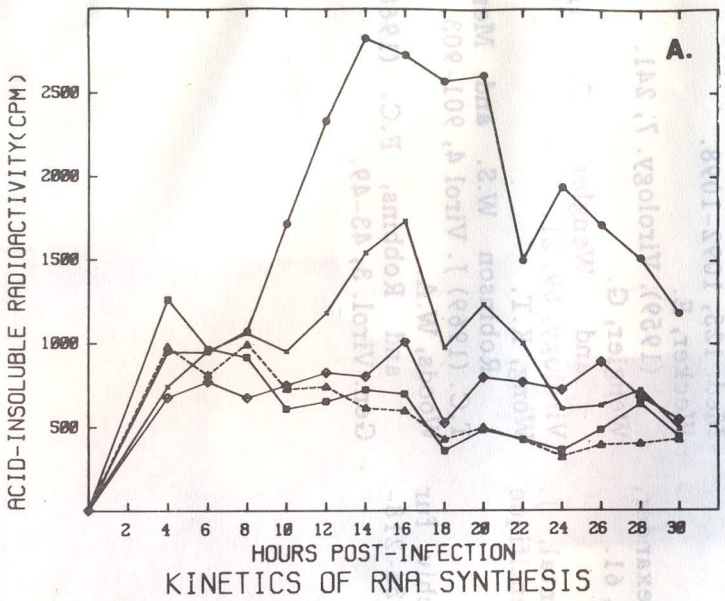

Figure A A composite comparing RNA synthesis for different cytopathogenic BVDV strains in But cells.

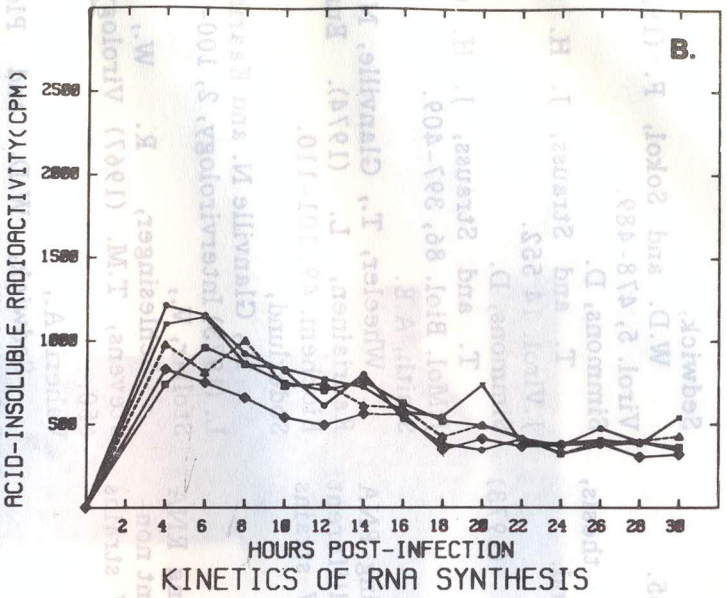

Figure B A composite comparing RNA synthesis for different non-cytopathogenic BVDV strains in But cells.