# A NUCLEIC ACID ANALOGUE DEPENDENT ANIMAL VIRUS*

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We have been studying the properties of infectious bovine rhinotracheitis (IBR) virus, a virus probably related to herpes (Armstrong, et al., 1961). It was observed that IBR virus, like herpes, produces plaques in the presence of specific antiserum, suggesting cell to cell transfer of virus. To evaluate the contribution of infectious virus to plaque formation under these conditions, two nucleic acid analogues, 5-bromodeoxyuridine (BUDR) and 5-fluorodeoxyuridine (FUDR), were employed as inhibitors of virus synthesis. In the course of this work it was found that virus produced in the presence of analogues becomes dependent on them for plaque production. The data documenting this observation is presented in this preliminary report.

The bovine kidney cell line, nutrient media, and origin of the cloned virus strain used have been described by Madin and Darby (1958) and Stevens and Chow (1959). The liquid medium contained lactalbumin hydrolysate (0.5%) in Earle's saline supplemented with 5% lamb serum (La-S). Six ml of medium were used per 2 oz French square bottle for growth and maintenance of cells.

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Plaque production was also carried out in the same liquid medium except that bovine antiserum (1:100) was added. A 1:500 dilution of this serum inactivated 150 out of 200 plaque-forming units (PFU) of IBR virus in one hour at 37°C. The effectiveness of the antiserum was confirmed by the absence of secondary plaques which are easily recognized when present. Plaques were stained as described by Holland and McLaren (1959) 48 to 72 hours after adsorption of virus. Titers were based on at least duplicate determinations. Analogue concentrations were BUDR, 100 μg/ml and FUDR, 10 μg/ml. Cell growth, virus adsorption and production, and plaque production were carried out at 37°C. Further details of the plaque procedure, a kinetic analysis of the system, and certain properties of the virus will be described in subsequent publications.

When single (24 hour) cycles of virus growth were carried out in antiserum-free La-S medium in the absence and presence of BUDR and FUDR, the yield of IBR virus was reduced from a control level of 116-580 PFU per infected cell to only 0.08-0.6 PFU per cell. Titrations were carried out in the standard manner, i.e. in the absence of analogues. Control- and analogue-grown virus preparations were then compared for their relative plating efficiency in the absence and presence of analogues. The results are given in Tables I and II.

Virus produced in the absence of analogues plated with equal efficiency whether or not the analogues were present in the assay system (Table I). In contrast, virus produced in the presence of analogues plated with a 37-87 fold greater efficiency when these compounds were present (Table II). Thus, for most of the virus produced in the presence of analogues, plaque production is analogue dependent.

Clearly, a virus population with altered characteristics is produced in the presence of analogues. Whether analogue dependent virus arises as a result of an induced change or selection remains to be determined. The fact that 5-10 PFU of dependent virus are produced per infected cell in a single cycle of growth in the presence of analogues does suggest that the change to dependence is induced.

TABLE I

The Effect of Nucleic Acid Analogues on Plaque Production by IBR Virus Grown in La-S Medium

| Titrating medium | Relative plaque counts Exp. | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| La-S + Antiserum | 74 | 108 | 88 |
| La-S + Antiserum + BUDR + FUDR | 75 | 122 | 84 |

In each experiment 0.2 ml of an appropriate dilution of virus grown in La-S medium was added to replicate monolayers. Monolayers which were to receive analogues were pretreated with 100 µg/ml of BUDR for 36 hours prior to virus adsorption. Adsorption was permitted for one hour after which La-S medium plus bovine antiserum (1:100) with or without analogues was added. The analogue concentrations were BUDR, 100 µg/ml and FUDR, 10 µg/ml. Plaque counts were determined after 2 days in control cultures and after 3 days in cultures containing analogues. At these times plaques were similar in size.

TABLE II

The Effect of Nucleic Acid Analogues on Plaque Production by IBR Virus Grown in La-S Medium Containing Analogues

| Titrating medium | Relative plaque counts Exp. | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| La-S + Antiserum | 230 | 120 | 220 |
| La-S + Antiserum + BUDR + FUDR | 20,000 | 6500 | 8240 |

These experiments were carried out in a manner identical to that described in Table I except that the virus preparation was produced in La-S medium containing BUDR and FUDR.

In formulating an explanation for these findings, two facts are of prime importance; first, that FUDR interferes with deoxyribonucleic acid (DNA) synthesis by blocking thymidylate synthesis, and second, that BUDR can be incorporated into DNA in place of thymidine and to some extent mitigate the toxic effect of FUDR (Levintow and Eagle, 1961). It is also known that BUDR can be incorporated into virus DNA (Stahl, et al., 1961). It seems reasonable to assume, therefore, that BUDR is incorporated into IBR virus nucleic acid when BUDR and FUDR are present during virus reproduction. Given this assumption, analogue dependence could be explained by proposing that BUDR-containing virus replicates more effectively in the presence of BUDR than in the presence of thymidine. Such dependence could manifest itself at many stages of the reproductive cycle; however, the step involving replication of nucleic acid seems most logical. Studies on the origin of dependent virus and on the nature of analogue dependence are being continued.

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