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TO KHENG, JUSTIS AND HUI LENG

ORF VIRUS: ITS POLYPEPTIDES AND CELL CYCLE

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ABSTRACT

To obtain information on the biology of orf virus *in vitro*, a cell culture system was established. Orf virus grew best in primary lung and testis cells of ovine and bovine origin compared with MDBK, MDCK and RK 13 cell lines and the highest yields of virus were obtained when growth medium was supplemented with 0.5% lactalbumin hydrolysate (LAH). Over 95% of the progeny virus remained cell-associated and thus virus yields could be enhanced by freezing and thawing and sonication.

The plaque and quantal assay, as compared with fluorescent focus assay, proved to be the simplest means of assaying viral titres although a period of a week was usually required before the results of the assay could be read.

One-step growth experiments showed that orf virus DNA synthesis in primary bovine testis cells began at 4-8 hr post infection (p.i.), rapidly increased from 12-14 hr and continued for up to 30-35 hr after infection. New infectious particles could be detected at 16-18 hr p.i. and rose to a maximum by 48 hr. In comparison, vaccinia virus DNA synthesis in the same cell system began at 3 hr p.i. and was almost complete by 12 hr. Orf virus DNA synthesis was inhibited by cytosine arabinoside, bromouracyldeoxyribose and hydroxyurea but was not inhibited by adenosine arabinoside.

A comparison of the virion polypeptides of 6 New Zealand orf isolates was undertaken. Sodium dodecyl sulphate-polyacrylamide gel electrophoretic analysis of virion polypeptides revealed similar profiles for 5 isolates while the remaining isolate gave a profile distinct from the others. Treatment of virions with nonidet P-40 and 2-mercaptoethanol solubilised 12 polypeptides which were believed to be surface components of the virion. One polypeptide with a molecular weight of 38.5K was believed to be a major component of the characteristic surface tubule of the virion.

The sequential appearance of virus-induced polypeptides in bovine testis cells was also determined. Bovine testis cells were infected at 15-25 pfu/cell and pulse-labelled with ^{35}S-methionine for 2 hr at various times after infection. Only 1 or 2 labelled polypeptides were detected within 8 hr p.i. Most of the virus-coded polypeptides were detected at 10 hr p.i. and thereafter. Most of the detectable polypeptides were also found in labelled whole virus preparations. A virion polypeptide with a molecular weight of 93K was believed to be a product of post-translational protein cleavage or other modification.

Another aspect of the orf virus/cell cycle investigated was the virus-specific cytopathic effect. Early cell rounding, seen as early as 2 hr p.i. in orf virus-infected bovine testis cells, was inhibited by cycloheximide (300 µg/ml) and actinomycin D (5 µg/ml). It was not inhibited when actinomycin D was added at 2 hrs p.i. Also, inhibition of

early cell rounding was seen when α-amanitine (10 µg/ml) or tunicamycin (2 µg/ml) was used. Ultraviolet irradiation of virus inoculum at a dose of 0.38 x $10^3 J/m^2$ reduced early cell rounding by 50% while 50% viral infectivity was reduced by a dose of 0.39 x $10^2 J/m^2$. A comparison of these results with those obtained by others indicate that early cell rounding is mediated by a gene product expressed early in infection, rather than by some toxic virion component(s).

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