The Isolation and Partial Characterisation of a Cytomegalovirus from the Brown Rat, *Rattus norvegicus*

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With 4 Figures

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Summary

This report describes the isolation and partial characterisation of a cytomegalovirus (CMV) from the wild brown rat (*Rattus norvegicus*). The isolate was sensitive to ether and heat treatment at 56 °C/30 minutes, and had a characteristic herpes virus morphology. In rat embryo fibroblast cells, new virus was detectable by 18 hours after inoculation. The cytopathic effect consisted of a focal rounding of cells which developed to involve the entire monolayer. Inoculation of newborn rats produced mortality of 67—75 per cent which was reduced to 33—46 per cent by intra-peritoneal inoculation or tissue culture passage. Highest virus concentrations were found in the liver and spleen 14 days after inoculation. The virus was also pathogenic (to a lesser extent) to newborn BALB/c mice. It was related to the Osborn strain of mouse CMV by fluorescent-antibody testing but distinct by the serum-neutralisation test.

Introduction

Cytomegaloviruses are members of the Herpetoviridae (Andrewes, Pereira, and Wildy, 1978) which are characterised by having a DNA genome enclosed in an icosahedral capsid and surrounded by a pleomorphic inner membrane. A marked species specificity has been observed with CMV from all species so far studied, although this specificity is not so pronounced in cell culture (Lussier, 1975).

Acidophytic intranuclear inclusions were first recorded in the submaxillary glands of rats by Thompson (1932). It was suggested that the inclusions were of a viral origin by analogy with a similar inclusion disease in guinea pigs (Cole and Kuttner, 1926). Similar inclusions in the salivary glands and kidneys of rats have been found by other investigators (Kuttner and Wang, 1934; Syverton and Larson, 1947; Lyon et al., 1959; Rabson et al., 1969). Despite this there appears to have been only one reported isolation of a CMV from a rat, namely,
the Panamanian roof rat, *Rattus rattus* (Rabson et al., 1969). The mouse CMV isolated by Smith (1954) has been suitable for much experimental work but for certain physiological experiments involving embryo culture (to be published elsewhere) it was desired to use a rat CMV.

This paper describes the isolation of a CMV from a wild rat and some of its *in vivo* and *in vitro* properties. In addition, some biological and serological properties were compared with those of the Osborn strain of mouse CMV.

**Materials and Methods**

**Animals**

Specific pathogen free (SPF) Sprague-Dawley rats were supplied by the breeding unit of the Clinical Research Centre (CRC) Harrow. The breeding colony has been shown to be free of Kilham Rat Virus, Minute Virus of Mice, Mouse Hepatitis virus, *Mycoplasma pulmonis*, *Pasteurella pneumotropia*, *Reovirus* type 3, *Sendai* Virus, and Toolan H-1 Virus.

For the virus isolation attempts 22 rats from three sources were used; Gunn rats from the Royal Postgraduate Medical School, Sprague-Dawley rats from the conventional holding area of the CRC, and wild rats collected by the Environmental Health Department of the Harrow Borough Council. From the last source only animals that were thought to have died recently were used. Of the three cadavers used, two died of warfarin poisoning and one from a blow to the head; one was about 19 days pregnant having 8 foetuses and one resorption site.

All mice were SPF of the BALB/c strain because of its known susceptibility to mouse CMV.

**Virus Isolation Procedure**

The sub-maxillary gland was selected as the organ for attempted virus isolation. At post mortem one gland was fixed in formal saline, processed by standard histological methods, and stained with haematoxylin and eosin. The other gland was finely chopped in virus transport medium (Eagle's basal medium plus 10 per cent calf serum [FCS] 100 IU/ml sodium penicillin, 100 µg/ml streptomycin sulphate, 50 µg/ml Amphotericin B) and frozen at -70°C. Frozen material was subsequently thawed and inoculated intra-cerebrally into 1—2 day old SPF rats which were then examined regularly for signs of infection.

**Mouse CMV Preparation**

The Osborn strain of mouse CMV was supplied by Professor C. A. Mims, Guy’s Hospital Medical School, London. The virus had either been maintained in a virulent state by *in vivo* passage and harvesting of sub-maxillary glands (oP²/sg) or modified by passage in mouse embryo fibroblast cultures (oP₁/te).

**Cell Cultures**

Rat (REF) and mouse (MEF) embryo fibroblast monolayers were prepared by standard methods using 5 × 10⁶ cells/ml in Dulbecco’s modification of Eagle’s minimum essential medium (MEM) (with 5 per cent FCS and 100 IU/ml penicillin and 100 µg/ml streptomycin sulphate) to seed the cultures into 2 oz. medical flat bottles for virus isolations and growth-curve experiments or into “Costar” 24 well tissue-culture plates (Costar, Cambridge, Mass., U.S.A.) for plaque assays.

**Virus Assays**

Ten-fold dilutions of samples were made in Eagle’s MEM without added serum and then 0.2 ml was inoculated in duplicate onto confluent REF or MEF cells. Virus was allowed to absorb to cells for one hour at 37°C, rocking the plates every 15 minutes. The cells were then overlaid with Eagle’s MEM containing 5 per cent FCS and 1 per