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The Early Detection of Picodnavirus ×14 by Immunofluorescence* (33397)

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There are now a large number of small DNA-containing animal viruses which by analogy with the RNA-containing picornaviruses have been called "picodnaviruses" (1). The known physical and chemical properties of members of this group have been tabulated recently (2). To date, only rodent picodnaviruses are known to be capable of autonomous replication in susceptible cells, while the human and simian members appear to be unable to multiply unless host cells are also infected with adenovirus (3–5). The ×14 is a rodent picodnavirus serologically related to the H_3 hamster osteolytic virus (6). The ×14 exhibits a $T = 3$ pattern of icosahedral symmetry similar to that of K rat virus (7, 8). Because of its similarity to the defective picodnaviruses (adeno-associated satellite viruses), ×14 may serve as a useful model of an autonomous small DNA virus. However, to date it has been impossible to detect ×14 virus with certainty until 3 or 4 days after inoculation of susceptible host cells. The use of fluorescein labeled antibodies to follow the growth cycle of satellite virus in the presence of its helper (9) led us to apply similar methods in the present experiments to the possible early detection of ×14 virus.

Materials and Methods. A strain of ×14 virus kindly supplied by Dr. F. E. Payne was grown in primary tissue cultures prepared from Sprague-Dawley rat embryos as described previously (7). Confluent monolayers on coverslips in Leighton tubes were inoculated at a multiplicity of approximately 1 $TCID_{50}$/cell. The cells were harvested at various times after infection, frozen and thawed three times and assayed for hemagglutinating activity (HA) and infectivity. Companion coverslip cultures were fixed in acetone for 10 min and stained by the direct fluorescent antibody technique as described previously (9). The hemagglutination pattern test was carried out in a plastic plate using 0.4% guinea pig erythrocytes (10). For convenience, the plates were held at 4° overnight before reading although equally high titers were obtained when the test was carried out at room temperature. The HA titers were expressed as the reciprocal of the highest

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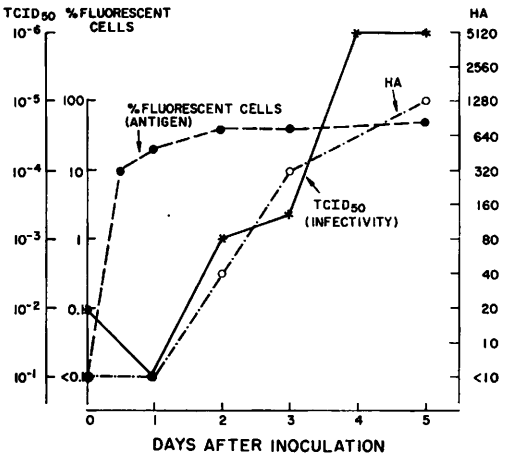

FIG. 1. Growth curves of ×14 virus infectivity ($TCID_{50}$), capsid antigen (% fluorescent cells) and hemagglutinin (HA) during a single cycle of infection.

dilution causing agglutination of 50% red cells. Antiserum against ×14 virus was prepared in guinea pigs using the following schedule: 1 ml of virus suspension containing approximately 10^6 $TCID_{50}$ was inoculated subcutaneously and followed by an identical booster inoculation 4 weeks later. Serum was collected 1 week following the booster injection (HI titer = 5120) and the globulin fraction was conjugated with fluorescein isothiocyanate as described previously (11). Infectivity titrations were carried out in tubes as described by Payne *et al.* (6).

Results. The results of following a single growth cycle of ×14 virus by hemagglutination, infectivity assay, and production of capsid antigen are shown in Fig. 1. The HA was first detectable at low levels (1:40) 2 days after inoculation. High HA titers (at least 1:1280) were not obtained until 4 or 5 days after inoculation. Infectious virus was barely detectable 2 days after inoculation and reached a maximum titer at 4 or 5 days. By contrast, viral antigen was clearly detectable by immunofluorescence in 10% of cells 12 hr after infection. The antigen was nuclear and stained brightly (Fig. 2a,b). Its demonstration was prevented by prior treatment of coverslips with unlabeled ×14 globulin. Early staining patterns did not appear to involve the nucleolus. By 24 hr after infection, the antigen often appeared to be concentrated in a brilliant perinuclear ring either as a continuous band or in discrete patches (Fig. 2c,d). By 48 hr, many brilliantly staining nuclei were observed in about 20–40% of the cells (Fig. 2e). The patterns of antigen distribution appeared very similar to those of SV40 and satellite virus antigens (12).

Discussion. Previous studies have shown that it is possible to detect satellite virus by immunofluorescence as early as 12 hr after infection (9). However, electron microscopic particle counts indicate that substantial yields of satellite particles (approx 10^{10}/ml) can be detected with confidence at this stage. The present experiments show that early detection of another picodnavirus is also possible with fluorescent antibody staining. In the case of ×14, however, fluorescent antibody techniques appear to be the only sure means of detection at this early stage. In 4 separate experiments in which companion cultures yielded brilliant fluorescence in 20% of the cells, electron microscopic particle counts were negative, that is, the concentration of virions was less than 10^7 ml. By contrast, 3 days are needed for detection by HA and infectivity of ×14 virus, and previous reports using acridine orange staining and

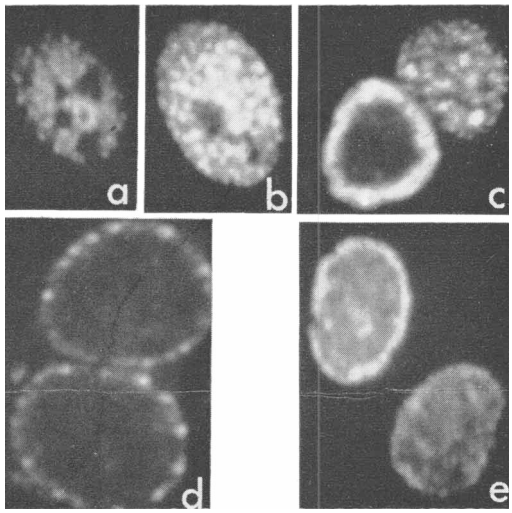

FIG. 2. Demonstration of ×14 capsid antigen by immunofluorescence in nuclei of rat embryo cells: (a) and (b) 12 hr after infection; (c) and (d) 24 hr after infection; (e) 48 hr after infection.

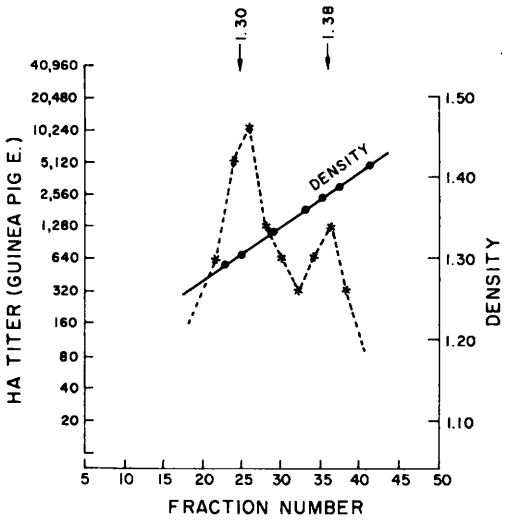

FIG. 3. A partially purified sample of ×14 virus was centrifuged to equilibrium in a cesium chloride density gradient. Droplet fractions were collected, their refractive indices were measured and buoyant densities were computed. Fractions were also assayed for hemagglutinating activity (HA). Infective virus banded at 1.38 g/cm^3, empty capsids at 1.30 g/cm^3; both peaks had high HA activity.

electron microscopy indicate 3 or 4 days are necessary (7). Hemagglutination is, however, the method of choice in detecting ×14 after purification in isopycnic density gradients [(6) and Fig. 3]. It is of interest that the density of ×14 (1.38 g/cm^3) is identical to that reported for K rat virus (2), and similar to the defective satellite viruses types 1, 2, and 3 (1.38–1.40 g/cm^3) (4, 5). However, the reports cited indicate that these genomes are considerably larger than that of K rat virus (approximately 3×10^6 daltons as compared with 2×10^6 daltons for K rat).

Continued comparative studies with picodnaviruses such as K rat and ×14 can be expected to throw more light on the nature of the deficiency of satellite DNA—a deficiency which is apparently not due to a paucity of nucleic acid.

Summary. Using fluorescein-labeled anti-×14 globulin, capsid antigens of ×14 virus can be detected in infected cells as early as 12 hr after infection. Biological techniques and electron microscopy require from 2 to 4 days for positive identification of the agent.

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