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SHORT COMMUNICATION

DIFFERENCE BETWEEN ONCOGENIC AND NONONCOGENIC HUMAN ADENOVIRUSES IN PLAQUE FORMING ABILITY IN AN ESTABLISHED CALF KIDNEY CELL LINE

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It is well known that human adenoviruses form plaques on various cultured human cells (Kjellén, 1961; Rouse et al., McBride and Wiener, 1964; Shimojo et al., 1966) and grivet monkey kidney cells (Tytell et al., 1962). During work on the growth characteristics of human adenoviruses in calf kidney cells (CKT) established in our laboratory (Kimura et al., 1968), we made the interesting observation that nononcogenic viruses form clear plaques on CKT cells, while no plaques are formed by viruses showing oncogenicity in hamsters.

The following twelve strains of adenovirus were used; type 1 (Adenoid 71), 2 (Adenoid 6), 3 (G.B.), 5 (Adenoid 75), 6 (Tonsil 99), 7 (Gomen), 8 (Ishimine), 9 (Hicks), 12 (Huie), 13 (A.A.), 14 (Dewit) and 18 (D.C.). Stock virus preparations were obtained by collecting fluid of infected HeLa cells.

Plaque assay was performed on monolayers of CKT cells (5×10^5 cells) in tissue culture bottles ($5\times3\times3$ cm) by inoculating 0.2 ml of serial ten-fold dilutions of stock virus preparations. After 3 hours adsorption at 37 C, the infected cultures were washed with YLE and overlaid with 5 ml of nutrient agar containing modified Earle's salt solution (Kjellén, 1961; Tytell et al., 1962). After 5 days incubation, the bottles were overlaid with an additional

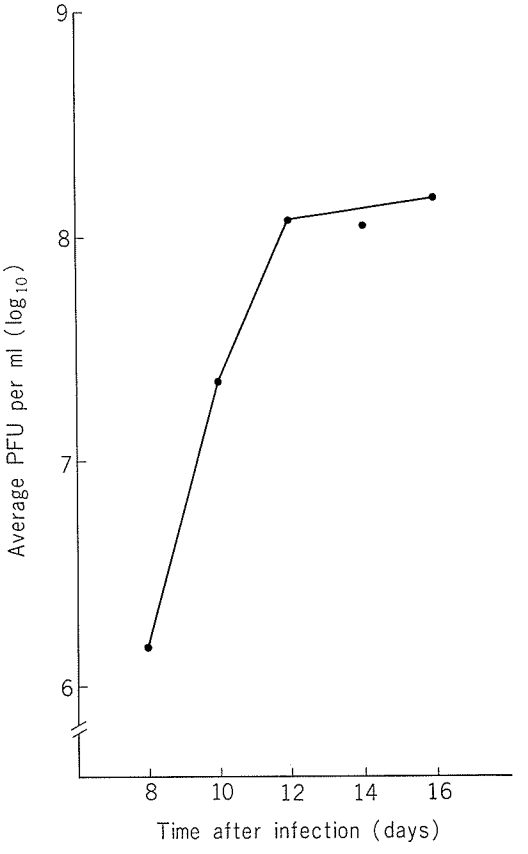

FIGURE 1. *Relationship between Plaque Number and Time after Infection of Adenovirus Type* 1.

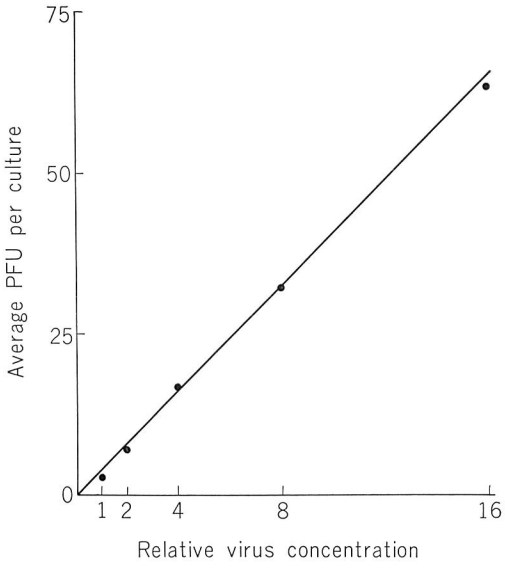

FIGURE 2. *Relationship between Number of Plaques and Relative Concentration of Adenovirus Type* 1.

5 ml of nutrient agar and incubated for a further 7 days. Then 12 days after infection 0.3 ml of neutral red solution (1 : 1,000) was added and cultures were kept in a dark place overnight at room temperature before counting

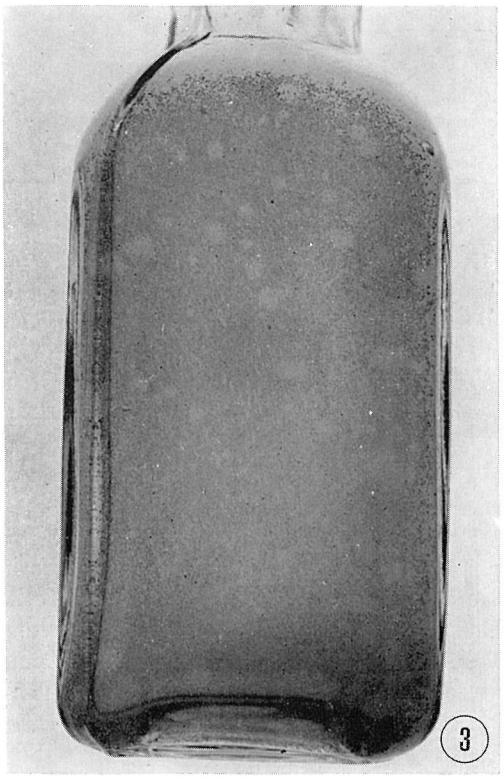

FIGURE 3. *Plaques of Adenovirus Type* 1 *in CKT Cells.*

TABLE 1. *Plaque forming ability of human adenoviruses on CKT cells*

Type	Inoculum ($TCID_{50}$/0.2 ml/bottle)[a]	Plaque count per bottle	Plaque forming ability (PFU/ml)	Oncogenicity
1	6×10^{-2}	24,28,31	+ (1.4×10^{7})	none
2	6×10^{-2}	22,17,19	+ (1.1×10^{7})	none
3	60	0	−	weak
5	2×10^{-1}	68,52,55	+ (2.9×10^{7})	none
6	2×10^{-2}	27,29,28	+ (1.4×10^{8})	none
7	60	0	−	weak
8	6	18,14,16	+ (8.0×10^{3})	none
9	6×10^{-1}	17,20,18	+ (9.1×10^{3})	none
12	60	0	−	high
13	6×10^{-1}	47,55,45	+ (2.5×10^{4})	none
14	60	0	−	weak
18	60	0	−	high

a : $TCID_{50}$ were estimated with HeLa cells on the 7th day after virus-infection.

their plaque number. Plaques of adenovirus type 1 were observed after 8 days infection and a constant plaque number was obtained after 12 days (Fig. 1). Under these conditions there was a linear relationship between the number of plaques and the relative concentration of adenovirus type 1 (Fig. 2). The appearance of plaques produced by type 1 in CKT cells is shown in Fig. 3.

It was found that on CKT cells human adenovirus types 1, 2, 5, 6, 8, 9 and 13 produced plaques but types 3, 7, 12, 14 and 18 did not, even though inocula of the latter series were larger (Table 1). In viral growth experiments we also found that types 1 and 5 could grow in CKT cells, while types 3, 7 and 12 could not (unpublished data). Green et al. (1967) reported that adenoviruses could be classified into three groups according to their plaque size in KB cells. However, we found that human adenoviruses could be classified into 2 groups with respect to their plaque forming ability in CKT cells and the serotype which was reported to have oncogenicity could not produce plaques.

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