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PLAQUE FORMATION BY ADENOVIRUS 7 IN HUMAN DIPLOID CELL STRAINS

L. KUTINOVÁ, V. VONKA

Department of Virus Biology, Research Institute of Immunology, Prague, Czechoslovakia

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Summary. — Adenovirus 7 (Gomen) induced plaque formation in human diploid cell strains. Plaques appeared on the 8th or 9th day after inoculation and their number increased till the 30th day. The number of plaques was proportional to the virus input. No difference was observed between the adeno 7 (Gomen) and the hybrid SV 40-adeno 7 (SP 2) viruses as regards plaque morphology, time development, and relation of plaque counts to virus input.

The most frequently employed host systems for the cultivation of human adenoviruses are continuous cell lines of human origin, such as HeLa, or KB (e.g. Trentin *et al.*, 1962; Green *et al.*, 1967), and primary human embryo kidney cells (e.g. Huebner *et al.*, 1963; McBride and Wiener, 1964). The latter system, which seems to be the most sensitive one, has also been successfully employed in the plaque technique with adenoviruses (e.g. Shimojo *et al.*, 1966; McAllister *et al.*, 1966). Because of difficulties with the regular supply of these cells we tried human diploid cells as an alternative system.

Two strains of human diploid cells, denoted LEP-12 and LEP-14, derived from the lungs of three-month-old human embryos and cultivated as described previously (Kutinová *et al.*, 1967), were employed. In the present experiments the cell strains were in the 14th—26th passage. The number of cells exhibiting alterations of karyotype at these passage levels never exceeded 10%. We found that adeno 3 and adeno 7 prototype viruses and the SV 40-adeno 7 hybrid virus (SP 2) replicated well in these cells eliciting a cytopathic effect typical of adenoviruses. For the plaque technique confluent cultures grown in Blake bottles were used. The cultures were inoculated with 0.1 ml volumes of serial tenfold dilutions of virus. After 1 hour of incubation at 36° C they were overlayed with 20 ml EPL medium (Michl and Řezáčová, 1966) containing 1% Difco Bacto agar, 2% calf serum, 40 mM $MgCl_2$, 0.15% $NaHCO_3$ and antibiotics. After 7—8 days of incubation, 10 ml of second overlay containing 0.01% neutral red was added.

Instances of plaques induced by adenovirus 7 (Gomen) can be seen in Fig. 1. The plaques were first seen on the 8th or 9th day after inoculation; their number increased consistently until day 30, when the cultures usually started to degenerate spontaneously. The increase in plaque count from day 12 to 28 is shown in Table 1. The plaque counts in cultures inoculated with the highest virus dilutions make it unlikely that the late increases in plaque count were due to the development of "secondary" plaques. A similar slow increase in plaque count was observed by Kjellén (1961) in HeLa cells infected with adenovirus types 4 and 5. The relationship between plaque count and virus input (Fig. 2) shows that over the range tested the number of plaques was

Table 1. Increase in adenovirus 7 plaque counts in LEP cells during incubation

Virus dilutions	Plaque counts on the indicated days after inoculation*			
	12	15	20	28
10^{-2}	98	D	D	D
10^{-3}	6	14	50	99
10^{-4}	0	1	6	20

* Mean counts from three cultures.
D = degeneration of the cultures.

proportional to the virus input. This indicates that the plaques were initiated by single infectious particles.

No significant difference in plaque morphology, development with time, or relationship between plaque count and virus input was observed between

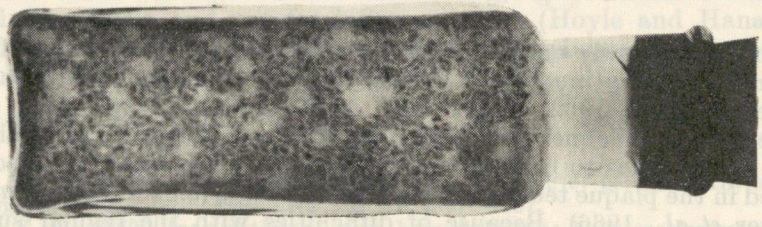

Fig. 1.
Plaques induced in LEP cells by adenovirus 7 (Gomen); 25 days after inoculation

adenovirus 7 (Gomen) and the SV 40 adeno 7 hybrid (SP 2) virus. An analysis of the progenies of single plaques elicited by the latter virus was presented elsewhere (Kutinová and Vonka, 1968).

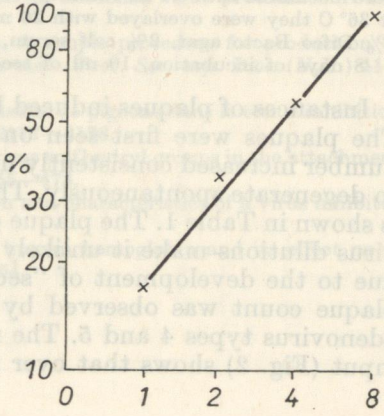

Fig. 2.
Relationship between plaque count and concentration of virus inoculum
Abscissa: relative concentration of virus inoculum; ordinate: plaque count per cent

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