

30° C for 1 week. The growth was estimated as total cell count using a Thoma chamber.

I am grateful to Dr. D. S. Davies for his interest.

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<sup>1</sup> Foster, J. W., *Antonie van Leeuwenhoek*, **28**, 241 (1962).

## VIROLOGY

### Small-plaque and Large-plaque Variants of Papova Virus SV<sub>40</sub>

PLAQUE size constitutes an important genetic characteristic of many viruses, including polyoma virus<sup>1-3</sup>. We have shown the existence of large-plaque (*LP*) and small-plaque (*SP*) variants for another Papova virus—*SV*<sub>40</sub>.

The plaque-forming ability of nine strains of *SV*<sub>40</sub> was investigated: *A-426* (ref. 4), 128, 129, 130, 131 (all were isolated from *M. rhesus* kidney tissue cultures and kindly supplied by Dr. Deichman), 261 (isolated by Dr. Deichman from a hamster tumour induced by *SV*<sub>40</sub>), *MR-1*, *MR-2* (isolated in this laboratory from *M. rhesus* kidney tissue culture) and *I-1* (isolated from hamster tumour formed by *SV*<sub>40</sub>). All viruses were propagated in green monkey kidney tissue cultures. Plaques formed by these different virus strains were compared by inoculating green monkey kidney cell culture in 50-ml. bottles with about 50 p.f.u. in 0.1–0.2 ml. After 2 h adsorption at room temperature, the cell monolayer was overlaid with nutrient agar using a modification of the technique of Stinebaugh and Melnick<sup>5</sup> ('Difco' agar, 1.5 per cent; ten-fold Earle's solution, 10.0 per cent; skimmed milk, 10.0 per cent; calf serum, 1.0 per cent; sodium bicarbonate, 0.4 per cent; neutral red, 0.0017 per cent; in de-ionized water). The infected tissue cultures were incubated at 37° C. The timing of plaque appearance was followed and their size measured on a given day of incubation.

The diameters of the plaques formed by two virus strains on the fourteenth day after inoculation are shown in Table 1 (all plaques formed in the bottles were measured). Though great variability of plaque size is characteristic of *SV*<sub>40</sub>, strain *A-426* predominately formed larger plaques, while strain 128 formed smaller ones. The difference could easily be seen without special measurement. Moreover, the plaques of strain *A-426* appeared 2–7 days earlier than did those of strain 128 (the exact interval varied for different batches of tissue culture cells).

Table 1. COMPARISON OF THE SIZE OF PLAQUES FORMED BY TWO *SV*<sub>40</sub> STRAINS ON THE FOURTEENTH DAY AFTER TISSUE CULTURE INOCULATION

Strain	No. of plaques measured	Percentage of plaques at the given diameters (mm)						Mean diameter (mm)
		< 0.5	0.5–0.7	0.8–1.0	1.1–1.5	1.6–2.0	> 2.0	
<i>A-426</i>	143	0.7	24.4	28.0	21.7	20.3	4.9	1.1
128	164	28.6	58.0	10.4	2.4	0.6	0.0	0.5

Investigation of the other seven strains of *SV*<sub>40</sub> showed that strain 130 was identical with strain 128. This was so with respect both to the rapidity of plaque formation and the size of plaques formed (these strains were therefore termed *SP* strains); the remaining six strains were identical with strain *A-426* (*LP* strains). Differences between *SP* and *LP* strains were constantly found in repeated assays, though they varied in degree.

It is possible that these differences between the two types of strains may be associated with the contamination of *SP* strains by a high concentration of a latent interfering virus. Simultaneous inoculation of tissue culture with equal doses of strains *A-426* and 128 had, however, no effect on the time of appearance or on the size of plaques induced by the former (*LP*) strain. Differences in plaque formation cannot, therefore, be accounted for by contamination by an interfering agent.

All the strains examined were cloned, and the cloned lines were passed four times in green monkey kidney cell culture. All cloned lines were identical in plaque-forming capacity with their parent strains.

To establish that plaque size was a genetic characteristic of virus particles, we investigated clones obtained from small and large plaques of *SP* and *LP* strains (Table 2). Whatever the size of the plaque used for cloning, the resulting clones did not differ from their parent strains. Plaque size was variable within each strain, but could not be accounted for by the viral genome.

Table 2. CHARACTERISTICS OF PLAQUE FORMATION BY CLONED LINES OBTAINED FROM LARGE AND SMALL PLAQUES OF LARGE-PLAQUE (*LP*) AND SMALL-PLAQUE (*SP*) STRAINS OF *SV*<sub>40</sub>

Strain	Original characteristic	Diameter of the plaque cloned (mm)	Number of clones	Clone characteristics*
<i>A-426</i>	<i>LP</i>	0.3–0.5	3	<i>LP</i>
		2.0–3.0	3	<i>LP</i>
129	<i>LP</i>	0.3–0.5	3	<i>LP</i>
		2.0–3.0	2	<i>LP</i>
128	<i>SP</i>	0.3–0.5	3	<i>SP</i>
		1.0–1.5	2	<i>SP</i>
130	<i>SP</i>	0.3–1.5	7	<i>SP</i>

\* Rate of appearance and the size of plaques were determined by comparison with those of *A-426* strain.

According to preliminary data, *SP* and *LP* strains do not differ in their sensitivity to agar polysaccharide and do not adsorb on to cells at different rates—as has been shown for polyoma virus variants<sup>6</sup>. Both *SP* and *LP* strains of *SV*<sub>40</sub> produced transformation in cultures of human and rat embryo fibroblasts<sup>7</sup>.

Further investigations should show whether it is possible to isolate *SP* variants from *LP* strain population and *LP* variants from *SP* strain population.

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## CYTOLOGY

### Microtubules in the Cytoplasm of Mammalian Platelets

OF late, and with increasing frequency, microtubules have been recognized as a cytoplasmic component in cells examined in thin section by electron microscopy (see review by Slautterback<sup>1</sup>). Their presence in the cytoplasm of human, guinea-pig and mouse platelets is recorded in this communication.

Using siliconized hypodermic needles and glassware and 3.8 per cent trisodium citrate (pH 7.4) as an anticoagulant, blood was collected from animals of both sexes. It was taken from the antecubital vein of eleven humans and, after an ether anaesthetic, by cardiac puncture from six guinea-pigs and from the retro-orbital sinus of eighty-four mice. That from six or twelve mice was pooled for subsequent processing. Aliquots of platelet-rich plasma, which had been separated from the blood by centrifugation, were fixed 2 h in 5 per cent glutaraldehyde<sup>2</sup> made up in Sørensen's phosphate buffer (pH 7.4), and were then subjected to further centrifugation. Platelet pellets so obtained were washed in the phosphate buffer, post-fixed 2 h in osmium tetroxide containing sucrose<sup>3</sup> (pH 7.4), then washed in water, dehydrated in ethanol, and embedded in 'Araldite'. Grey to pale gold thin sections