34, since antigen 3 of *S. anatum* is identical with that of group *E*₂ cells or of *A*(ϵ^{15}) cells but there is neither formation of antigen 34 nor loss of antigen 3 in *A*(ϵ^{34}).

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Virus Release from Single Cells of Mouse Parotid Tumours

In vitro studies have shown that cells from polyoma-induced mouse tumours in mass culture can release virus, and that the release can vary in different tumours^{1,2}. One of the factors responsible for this difference in release is the proportion of virus-releasing cells as measured by plating the tumour cells on mouse embryo monolayers, and determining the relationship between the number of tumour cells plated and the number of plaques². The present communication is concerned with a study on single cells from mouse parotid tumours grown in microdrops, in order to obtain information on: (a) the proportion of cells releasing virus by plating in microdrops, and (b) the virus release per cell.

The parotid tumours were induced by inoculation of polyoma virus into 2- or 3-day old mice, and the tumour cells were dispersed by trypsinization with a 0.25 per cent trypsin solution (trypsin 1-300). Virus release per cell was tested either by seeding the cells directly in microdrops, or by seeding in microdrops cells that had been in mass culture. In all cases, the medium consisted of 0.5 per cent lactalbumin hydrolysate in Earle's saline and 20 per cent horse serum.

Cell suspensions were diluted in medium to about 10^3 cells in a 50-mm. Petri dish, and these were then used for the single cell seedings. The single cells were distributed freehand in microdrops with micropipettes under a dissecting microscope in an enclosed box, maintained at 37° C., with a humidified atmosphere containing 10 per cent carbon dioxide. The microdrops were made in 50-mm. Petri dishes under silicone fluid (Dow 'Corning 200')³, and the Petri dishes with microdrops were kept in an incubator with a humidified atmosphere containing 10 per cent carbon dioxide. The presence of only a single cell in each microdrop was checked by observation with an inverted phase-contrast microscope.

For collecting the samples, each drop was sucked up with a micropipette, and the cell was then washed twice with new medium. The complete sample per cell thus consisted of three drops, which were added to 0.2 ml. of medium. The samples did not include cell-associated virus. When cells were tested after they had been in mass culture (5×10^6 cells seeded in a 50-mm. Petri dish) the cultures were washed twice with medium and the cells removed from the glass by trypsinization with a 0.25 per cent trypsin solution for 10 min. at 37° C. Not all the cells came off the glass by this procedure, and only those that were detached after 10 min. trypsinization were used for

the microdrops. The samples collected from the single cells were tested for plaque-forming units on mouse embryo monolayers as described previously⁴. The number of plaque-forming units was read at 11 days after inoculation of the monolayers, and in the case of *MP12* again at 16 days.

The proportion of single cells, from 6 parotid tumours, releasing virus under these conditions is shown in Table 1. Cells from two of these tumours were tested without previous mass culture, and the samples were taken at periods of 28-80 hr. after seeding the single cells. The highest proportion of releasing cells, 9/19, was found in tumour *MP2*, and this tumour had also given the highest release by cells in mass culture (haemagglutination titre of 2,048 per ml. on the day equivalent to that used for sampling from microdrops). In the other five tumours, virus release was detected in only 3 out of 172 cells tested. None of the single tumour cells was seen to divide under these conditions.

Table 1. PROPORTION OF SINGLE CELLS FROM SIX MOUSE PAROTID TUMOURS RELEASING VIRUS IN MICRODROPS

Tumour	Strain of mouse	No. of days in mass culture	No. of cells tested	No. of cells releasing virus	Release period (hr.)
<i>MP4</i>	<i>AKR</i>	0	16	1	72
<i>MP3</i>	<i>AKR</i>	0	13	0	80
<i>MP2</i>	<i>AKR</i>	2	19	9	28
<i>MP12</i>	<i>DBA/2</i>	3	26	1	28
<i>MP13</i>	<i>SWR</i>	4	76	1	36 and 72
<i>MP14</i>	<i>SWR</i>	4	41	0	36 and 72

Total No. of cells tested, 191; total No. of cells releasing virus, 12.

The number of plaque-forming units released per cell in tumour *MP2* during a 28-hr. release period is shown in Table 2. The release varied from 0 to 9 plaque-forming units per cell with an average of 5 for the virus-releasing cells. This figure is similar to that reported for the Rous chicken sarcoma⁵, and whether parotid tumour cells have a regular release per day is now being tested. The release from the other 3 positive cells (Table 1) in 3 other tumours were 17 plaque-forming units in *MP4*, 4 in *MP12*, and 2 in *MP13*.

Table 2. VIRUS RELEASE FROM SINGLE CELLS IN MICRODROPS DURING A 28-HR. PERIOD

No. of plaque-forming units per cell	0	1	2	3	4	5	6	7	8	9
No. of cells	10	0	1	2	2	1	1	1	0	1

No. of cells releasing virus, 9/19; average release of virus releasing cells, 5 plaque-forming units.

The above results provide further information on the existence of a difference in virus release from different tumours, and they also show that under the conditions of these tests in microdrops, single cells from polyoma-induced parotid tumours have at most only a low virus release per day.

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