Dissociation between Cell Conversion Induced by Mouse Sarcoma Virus and Production of Infectious Virus

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The mouse sarcoma virus isolate MSV-M (MLV) induces a morphological conversion in monolayers of mouse cells. Although the mechanism of this phenomenon is not clear, it is known that it requires simultaneous infection of each cell by MSV and its helper virus, the Moloney leukaemia virus (MLV: Hartley & Rowe, 1966). In order to investigate the dependence of cell conversion on virus multiplication, 5-fluorouracil was used to block this multiplication.

MSV-M (MLV) stocks were prepared from BALB/c mouse tumour extracts (Chenaille et al. 1967). These stocks contained defective MSV and MLV with competent MSV (O'Connor & Fischinger, 1968; Guillemain et al. 1968) in a ratio 3/150,000/1 (Bernard et al. 1968). Cell line 8828 derived from mouse embryonic lung was kindly supplied by Dr Freeman (Microbiological Associates, Bethesda, U.S.A.) and was used in all experiments: this supports virus growth as well as mouse-embryo fibroblasts but cell conversion is more readily detectable.

Two methods were used to study cell conversion. Method (a), 3 x 10⁶ cells were grown in 50 mm Petri dishes with minimal essential medium (MEM) + 10% calf serum to obtain a monolayer within 24 hr; after discarding the medium, a volume of 1 ml. of MEM + 5% calf serum was added with MSV to produce about 100 foci. After adsorption for 30 min. at 37°, 4 ml. of medium containing 5% calf serum and 5-fluorouracil were added: four dishes were used at each of several concentrations of 5-fluorouracil. After incubation at 37° for 5 days, foci of cell conversion were counted in each dish using an inverted microscope. The results were expressed as focus forming units/ml. (f.f.u./ml.). Method (b), for experiments using higher multiplicities of infection, 3 x 10⁶ cells were grown in Kahn tubes with MEM + 10% calf serum. Medium was discarded 24 hr later and MSV added under a volume of 2 ml. MEM + 5% calf serum containing 5-fluorouracil; several multiplicities of infection were used with several concentrations of 5-fluorouracil for each. Tubes were kept at 37° for 48 to 72 hr when overall conversion of the monolayer was observed under an inverted microscope.

After freezing and thawing three times, the cell suspension was clarified by centrifugation at 5000 g for 10 min. It was then diluted with MEM so that the concentration of 5-fluorouracil did not exceed 5 µg./ml. The MSV infectivity was estimated either by counting foci or by a simplified end-point technique in which 3 x 10⁴ cells (8828) were grown in Kahn tubes and infected 24 hr later with 1 ml. of MSV in MEM + 5% calf serum. The overall conversion was observed 72 hr later. It had been determined previously that the highest dilution at which overall conversion was detectable contained approximately 2 x 10³ f.f.u./ml. of MSV.

The rate of nucleic acid synthesis in cells was studied after labelling the cultures for 1 hr with 10 µc/ml. tritiated adenine or guanosine; nucleic acids were analysed according to the method of Schmidt & Thannhauser (1945) and the radioactivity collected on membranes was measured in a scintillation counter.

The action of 5-fluorouracil on cell conversion was studied following induction by MSV at various m.o.i. At m.o.i. below 10⁻³ f.f.u./cell, conversion in Petri dishes occurred as foci of
altered cells. Fig. 1 shows the percentage of foci as a function of the concentration of the inhibitor compared with a control without 5-fluorouracil. The relationship suggests a complex mechanism possibly related to the heterogeneity of MSV stocks. In this particular experiment, the m.o.i. was $2 \times 10^{-4}$ f.f.u./cell and the amount of 5-fluorouracil which gave 99.9% focus inhibition was about 1.0 μg/ml. For higher m.o.i. ($10^{-3}$ f.f.u./cell), the fully inhibiting dose

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**Fig. 1.** Percentage of foci counted at different concentrations of 5-fluorouracil in medium. The number of foci observed in the absence of 5-fluorouracil is taken as 100%. Multiplicity of infection: $2 \times 10^{-4}$ f.f.u./cell.

**Table 1.** Dissociation between cell conversion and production of infectious virus in presence of 5-fluorouracil

<table>
<thead>
<tr>
<th>(5-fluorouracil) μg./ml.</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>25</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular DNA</strong>*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>100</td>
<td>14.4</td>
<td>ND</td>
<td>10.0</td>
<td>ND</td>
<td>6.0</td>
<td>5.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Cellular RNA</strong>†</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>15.2</td>
<td>ND</td>
<td>11.4</td>
<td>ND</td>
<td>6.2</td>
<td>2.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>m.o.i. &lt; $10^{-3}$</td>
<td>CC</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PIV</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>m.o.i. = $7 \times 10^{-2}$</td>
<td>CC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PIV</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>m.o.i. = 1</td>
<td>CC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>PIV</td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tr>
</tbody>
</table>

* Rate of synthesis of cellular DNA expressed as percentage of control in absence of 5-fluorouracil.
† Rate of synthesis of cellular RNA expressed as percentage of control in absence of 5-fluorouracil. CC, cell conversion; PIV, production of infectious virus; ND, not done; m.o.i., multiplicity of infection.

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of 5-fluorouracil was higher but did not exceed 6 μg. 5-fluorouracil/ml. At m.o.i. between 1 and $7 \times 10^{-2}$ f.f.u./cell, cell conversion as studied in Kahn tubes appeared as an overall alteration of the cell sheet. The results show that at $7 \times 10^{-3}$ f.f.u./cell, cell conversion was still detected at a concentration of 25 μg. 5-fluorouracil/ml. whereas at a multiplicity of one
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conversion was present up to at least 200 µg./ml. (Table 1). On the other hand, no infective virus was produced at any multiplicity of infection above a concentration of 6 µg. 5-fluorouracil/ml.

There was no significant alteration during the time of cell multiplication in the presence up to 6 µg. 5-fluorouracil/ml. At 25 µg. 5-fluorouracil/ml. cell multiplication was inhibited and the rate of nucleic acid synthesis was reduced to 10% distributed equally between RNA and DNA (Table 1).

Thus, considering cell conversion detected by focus assay at low multiplicities of infection, the same dose of 5-fluorouracil appears to be inhibitory both for cell conversion and for virus multiplication. On the other hand, at high multiplicities of infection, when conversion relates to the whole cell monolayer, it may persist while virus multiplication is totally inhibited (Table 1).

This apparent contradiction in relation to multiplicity may be explained at low multiplicities in terms of cells which are converted directly but in too small a number to be detected. Conversion is visualized by focus formation which may result either from multiplication of previously converted cells or from re-infection of surrounding cells by MSV produced by originally converted cells. Hartley & Rowe (1966) have provided evidence in favour of the second hypothesis. Our results also favour this hypothesis since foci of cell conversion were not observed above a concentration of 6 µg. 5-fluorouracil/ml. which blocked virus multiplication without inhibiting cell growth, at least during the period of observation.

These results suggest that cell conversion induced by MSV is related to an effect which occurs before replication of virus. Also, conversion may be the result of an event coded by the virus genome since conversion can be observed in the presence of concentrations of 5-fluorouracil (200 µg./ml.) which inhibit almost completely the metabolism of cell nucleic acids. On the contrary, virus production requires host nucleic acid synthesis as already shown by Buck & Bather (1969).

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REFERENCES


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