Rescue of Rous Sarcoma Virus in Mixed Cultures of Virogenic Mammalian and Chicken Cells, Treated and Untreated with Sendai Virus and Detected by Focus Assay

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Rous sarcoma virus could be detected in mammalian tumours induced by it if these tumours were transmitted as fresh tissue suspensions into chicks (Svoboda, 1960, 1961). Detailed studies of this question (Svoboda, 1962, Šimkovič, Valentová & Thurzo, 1962; Svoboda et al. 1963) have established that close contact between virogenic mammalian cells and chicken cells is necessary for the formation of Rous sarcoma virus, and fusion between these cells is regarded as a most likely mechanism enabling the transmission of Rous sarcoma genetic material from the mammalian cells to the chicken cells (Svoboda et al. 1963). The finding that Sendai virus produces fusion between homologous (Okada, 1962) and heterologous (Harris & Watkins, 1965) cells stimulated us and others to make experiments which showed that the procedure also significantly increased the production of Rous virus in mixed cultures (Svoboda, Machala & Hložánek, 1967; Vigier, 1967; Yamaguchi, Takeuchi & Yamamoto, 1967). Rescue of SV 40 virus from mixed cultures of virogenic and indicator cells is also facilitated by treatment with Sendai virus (Gerber, 1966; Koprowski, Jensen & Steplewski, 1967; Watkins & Dulbecco, 1967).

The possibility of developing a direct assay system for detecting the rescue of Rous sarcoma virus in mixed cultures was investigated on the basis of the Rous sarcoma virus macrofocus assay (Dougherty & Simons, 1962). In these experiments Chinese hamster cells transformed with Rous sarcoma virus SCHMIDT RUPPIN strain (Hložánek, Donner & Svoboda, 1966) were used. No infectious Rous sarcoma virus was found in these cells, designated as RSCh, despite thorough testing for its presence.

Ten million RSCh cells, irradiated with 7000 r. (this dose prevents multiplication of cells) and 10⁷ primary chicken embryo fibroblasts from a Brown Leghorn strain were mixed, untreated or treated with 1000 haemagglutinating units (HAU) of u.v.-inactivated Sendai virus according to Okada’s procedure (Okada, 1962; Svoboda et al. 1967). Four million cells were seeded per plastic Petri dish in 4 ml. of medium 199, supplemented with 10% tryptose phosphate broth (Difco) and 5% calf serum. Eighteen hr later culture fluid was removed and 8 ml. of medium containing 0.8% agar (Difco) was added. After 5 to 7 days cultures were re-fed with 3 ml. of the same medium. Clearly discernible foci of transformed chicken cells developed after 12 to 15 days of incubation in a CO₂ atmosphere at 37.5°C. Plate 1, fig. 1, 2, show treated and untreated mixed cultures which were incubated for 19 days in order to allow the foci to develop to a very distinctive size. Foci formed in mixed cultures are always of variable size.

The specificity of the foci formed was tested. Eight foci were separately isolated from experimental cultures and subcultured. All regularly produced infectious Rous sarcoma virus, grew poorly, if at all, in the absence of the X-irradiated feeder layer of chicken cells and morphologically corresponded to chicken cells transformed by the
SCHMIDT RUPPIN strain of Rous sarcoma virus. Two of them were analysed karyologically, and typical microchromosomes were seen in all metaphases.

Foci were counted in three independent experiments (Table 1). In groups treated with Sendai virus the average number of foci exceeded the number in control plates by 40- to 50-fold. There was variation from one experiment to another, but the increase in control counts was always associated with an increase in experimental counts.

Table 1. Number of foci in mixed cultures of $2 \times 10^6$ RSCh and $2 \times 10^6$ chick embryo cells treated and untreated with Sendai virus

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 HAU Sendai virus</td>
<td>178, 187</td>
</tr>
<tr>
<td>None</td>
<td>2, 5</td>
</tr>
<tr>
<td>1000 HAU Sendai virus</td>
<td>217, 239</td>
</tr>
<tr>
<td>None</td>
<td>4, 7</td>
</tr>
<tr>
<td>1000 HAU Sendai virus</td>
<td>&gt; 300, &gt; 300</td>
</tr>
<tr>
<td>None</td>
<td>7, 11</td>
</tr>
<tr>
<td>1000 HAU* Sendai virus</td>
<td>0, 0, 0</td>
</tr>
</tbody>
</table>

* In this experiment mouse embryo cells were substituted for chicken cells.

In cultures where mouse embryo cells were substituted for chicken cells no foci developed. In three experiments $2 \times 10^5$ chick embryo cells were incubated for 45 min. at $37^\circ$ with 0.5 ml. freshly prepared filtrates of tissue culture fluid from RSCh cultures, treated with 1000 HAU of Sendai virus and seeded for focus assay. Neither foci of transformed cells nor production of Rous sarcoma virus were detected.

Focus assay, originally developed by Temin & Rubin (1958) for titration of Rous sarcoma virus can, therefore, be successfully adopted for the detection of virus rescue by the association of virogenic and sensitive cells. In addition, this assay enables direct recognition of factors enhancing the effect of association—such as cellular fusion produced by Sendai virus—and makes possible the further investigation of the whole phenomenon.

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REFERENCES


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EXPLANATION OF PLATE

Fig. 1. Numerous foci produced in the dish seeded with 4×10⁶ of the mixture (1:1) of RSCh and BLEF cells treated with 1000 HAU Sendai virus.

Fig. 2. Two foci developed in control untreated dish.