The Kinetics of DEAE-Dextran-induced Cell Sensitization to Transfection

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DEAE-dextran has commonly been found to more effectively enhance transfection when mixed with the virus nucleic acid before cell inoculation (mixed-inoculum method) than when it is used to pretreat the cells before inoculation (Pagano & Vaheri, 1965; Bachrach, 1966; Koch, Quintrell & Bishop, 1966; Pagano, McCutchan & Vaheri, 1967; Tovell & Colter, 1967). We have found, however, that the cells are only transiently sensitized to transfection by pretreatment, and that this method, optimally employed, can give much greater enhancement than the mixed-inoculum method. Part of these results have appeared in abstract form (Al-Moslih & Dubes, 1972).

Two picornaviruses were used: the BRUNHILDE strain of poliovirus type I on the eta and kappa (Chapin & Dubes, 1964) and LLC-MK2 lines of rhesus monkey kidney cells, a line of chimpanzee liver cells (Dr W. V. Hartwell), and the HeLa and KB lines of human carcinoma cells; and a rapid (r) mutant of the WALLACE strain of echovirus type 7 on cultures of eta and LLC-MK2 cells. This r mutant, isolated after selective passages on eta cells, produces plaques about 3 times larger in diameter than wild-type WALLACE virus. The cells were grown as sheets under medium S (Chapin & Dubes, 1964). From high-titre serum-free virus stocks which had been grown in eta cells under medium S minus serum, virus RNA preparations were made using three serial extractions with phenol at 0 °C (Gierer & Schramm, 1956). For enhancement, three procedures were tested: the mixed-inoculum method essentially as employed by Pagano & Vaheri (1965), the cell pretreatment and post-treatment methods. In each case, the cell sheet in a 60 mm Petri dish was incubated at 23 °C in 5 ml phosphate-buffered saline (PBS) of Dulbecco & Vogt (1954) minus CaCl2 and MgCl2 (medium A) for 15 min. Then the sheet was either pretreated with DEAE-dextran, or directly inoculated with the virus RNA either mixed or unmixed with DEAE-dextran. Each DEAE-dextran-pretreated cell sheet was washed 3 times, each time with 5 ml medium A, after pretreatment and before inoculation with RNA. In each case, unless otherwise noted, the RNA inoculum was incubated with the cell sheet at 23 °C for 15 min. For the post-treatment procedure, after incubation with the virus RNA, the cell sheet was washed once with 5 ml medium A. In all cases, each cell sheet was finally washed with 5 ml balanced salt solution of Hanks & Wallace (1949).

Representative data showing the considerably greater enhancement of transfection of eta cells afforded by the pretreatment method, as versus the mixed-inoculum method, are shown in Table 1. Essentially similar results were obtained in tests of the transfection of kappa, chimpanzee liver, HeLa, and KB cells by poliovirus RNA. Using LLC-MK2 cells, the pretreatment method also afforded greater enhancement of transfection by either poliovirus RNA or echovirus RNA than the mixed-inoculum method, but the differences were slight. The post-treatment method gave no enhancement.

Attempts were made, using eta cells and echovirus RNA, to increase the relative effectiveness of the mixed-inoculum method by testing various combinations of different concentrations of DEAE-dextran, temperatures, and durations of incubation of the mixed inoculum with the cells, as well as using PBS instead of medium A as RNA diluent. In none of these
Table 1. Comparisons of DEAE-dextran pretreatment and mixed-inoculum methods

<table>
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<tr>
<th>RNA*</th>
<th>Expt.</th>
<th>Number of plaques/plate†</th>
<th>Pretreatment method‡</th>
<th>Mixed-inoculum method§</th>
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<tr>
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<td></td>
<td></td>
<td>556</td>
<td>23</td>
<td></td>
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</table>

* For a given experiment, the inoculum RNA concentration was constant.
† Eta cell sheets were used. Counts above 350 plaques/plate were rough estimations.
‡ Washed cells were incubated with DEAE-dextran at 1 mg/ml at 23°C for 9 min (except 15 min for Expts. 1 and 2), washed three times with medium A, using 5 ml/wash, and then incubated with virus RNA at 23°C for 15 min.
§ Washed cells were incubated with a mixture of DEAE-dextran at 1 mg/ml and virus RNA at 23°C for 15 min.

combinations did the mixed-inoculum method approach the optimal pretreatment method in effectiveness.

The kinetics of cell sensitization by pretreatment was found to be markedly dependent on the concentration of DEAE-dextran and on the temperature (Fig. 1). The sensitization was followed by a marked desensitization process (Fig. 1). From the data of Fig. 1, and other experiments, several conclusions can be reached: the optimum temperature for pretreatment is dependent on the duration of the pretreatment. For example, with DEAE-dextran at 1 mg/ml, when the duration is 15 min, the optimum temperature is 18°C; but when the duration is <15 or >15 min, the optimum temperature is >18°C or <18°C, respectively. That is, these two variables show an inverse relationship. In sum then, the three variables DEAE-dextran concentration, temperature, and time are closely interrelated in determining the cell sensitization obtained in the pretreatment method; and it is meaningful to speak of the optimal value for one of these three variables only when the values of the other two variables are specified. As our standard system, we use 23°C for 9 min, where the optimum concentration of DEAE-dextran is 1 mg/ml. These values were obtained using eta cells and echovirus RNA, but similar values were obtained using other cell/RNA systems (Fig. 1).

The enhancement given by the pretreatment method was independent, or nearly independent, of pH of the pretreatment buffer over the range 6.4 to 8.0, but strikingly dependent on the buffer tonicity, with the optimal tonicity being approximately isotonic.

After very short pretreatment, the cells underwent further sensitization even after washing off the free DEAE-dextran; such further sensitization was shown both for poliovirus RNA
Fig. 1. Cell sensitization as a function of duration of pretreatment of cells with DEAE-dextran. Eta cells were used in A and B. Echovirus RNA was used in A, B and C; and poliovirus RNA in D. DEAE-dextran was at 1 mg/ml except in A. Pretreatment was at 23 °C in A, C and D, and at 37 °C in B. For B, C and D, the arithmetic mean plaque counts were normalized.

(Fig. 2) and echovirus RNA. This result indicates that some further step beyond mere attachment of the DEAE-dextran to the cells is required for full cell sensitization.

Cells which had been markedly desensitized by prolonged pretreatment with DEAE-dextran could be fully resensitized with fresh solution of DEAE-dextran.

Some of the early reports (see above) of low or no cell sensitization by DEAE-dextran were probably due to overlong incubation of the DEAE-dextran with the cells before inoculation with RNA; for example, Pagano & Vaheri (1965) pretreated their cells at 37 °C for 2 h. More recently, Koch & Bishop (1968) and Wentzky & Koch (1971) reported that cell pretreatment with DEAE-dextran gave moderately large enhancement of transfection, but still less than that given by the mixed-inoculum method.

To sum up, we conclude from our experiments that DEAE-dextran enhances transfection by sensitizing the cell, that this sensitization process consists of at least two steps, that sensitization is quickly followed by a markedly temperature-dependent desensitization process, and that, after desensitization, the cells are fully resensitizable by fresh solution of DEAE-dextran.
Fig. 2. Kinetics of further cell sensitization. Eta cell sheets were pretreated with DEAE-dextran at 1 mg/ml at 37°C for 1 min, then washed 3 times and incubated at 23°C for various periods of time before inoculating them with poliovirus RNA. The arithmetic mean plaque count data have been normalized.

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REFERENCES


Short communications


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