# Study of mechanisms of electric field-induced DNA transfection III

## Electric parameters and other conditions for effective transfection

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ABSTRACT Electric parameters, osmolality, temperature, and pH of the suspending medium and the growth phase of cells, etc., are known to influence the efficiency of the pulsed electric field (PEF)-induced DNA transfection of cells. PEF-induced transfection of *Escherichia coli* JM105 by plasmid DNA $PUC^{18}$, $PUC^{19}$, $PBR^{322}$, and PMSG has been used as a model system to establish quantitative relationships between these parameters and transfection efficiency. The main findings are summarized for experiments using unipolar square wave PEF. (*a*) For a given field strength (up to 6 kV/cm), the transfection efficiency (TE) was linearly dependent on the pulse width (up to 1 ms). (*b*) When field strength is fixed, Log [TE] correlated with the number of pulses applied. Similarly, when field duration was fixed, Log [TE] correlated with the number of pulses. (*c*) In the absence of $MgCl_2$, TE showed a maximal value at 50 mM sucrose and was reduced by several fold at lower and higher sucrose concentrations. Cell survival was nearly constant in the range 1-300 mM sucrose. (*d*) *E. coli* in the early and mid-exponential growth phases was more susceptible to PEF for DNA transfection than it was in the stationary phase. (*e*) For a given set of electric parameters, TE was the highest at neutral pH and was greatly reduced at acidic and alkaline pH. (*f*) Increasing the temperature from 0 to 37°C resulted in the reduction of TE by three orders of magnitude. This could reflect a rapid shrinking of pores at higher temperatures. (*g*) TE was inversely proportional to the square of the size of the plasmid DNA. By adjusting the above parameters to optimize transfection, a TE of $10^{10}$ $\mu g^{-1}$ DNA ($PUC^{18}$) has been recorded. Further improvement in percent cell transfection may be expected by a more exhaustive search of conditions than the present study has done.

## INTRODUCTION

Pulsed electric fields (PEF) of intensity, in kilovolts per centimeter and of duration in microseconds to milliseconds, when applied to cells in suspension, are known to cause many membrane phenomena; among these are changes in membrane permeability, induction of membrane fusion, insertion of molecules into lipid bilayers, appearance of large-scale morphological changes of cell membranes, and in severe cases, leakage of cytoplasmic contents and cell death (1, 2). A generally accepted term describing these effects of PEF on cells is electroporation (1-3). One important application of the electroporation is the introduction of foreign genes into living cells. Loading of SV40 DNA into erythrocyte ghosts was reported by Auer et al. (4) in 1976. The use of PEF for DNA transfection of the mouse L cells was reported by Neumann and co-workers (5, 6), who have shown that the loaded thymidine kinase gene was expressed. With its high efficiency and ease of use, the PEF method has quickly gained acceptance in many molecular biology laboratories for the routine DNA transfection of cells. Some examples of these applications are the transfection of mammalian cells (7-9), plant protoplasts (10-12), yeast spheroplasts (13), and bacteria (14-18).

Gene transfer into cells by PEF apparently is due to the transient permeabilization of the cell membranes (1, 2, 19-22). For efficient transfection, different forms of PEF have been tested. These PEF included rectangular single polarity (dc) pulses (17, 18), exponentially decaying dc pulses (5, 6), dc shifted oscillatory fields (23), and alternating electric fields (ac) (18, 24). Each waveform has advantages and disadvantages. For example, transfection by dc shifted oscillatory electric pulses or ac fields produces less cell death than other forms of dc pulses under conditions that give similar TE. However, oscillating fields generally require a longer exposure time than a dc field, and the temperature perturbation is consequently greater. In addition to waveforms, electric parameters and other factors also affect efficiency of transfection and cell survival (1, 21, 22).

Despite the widespread application of the PEF method, relatively few studies are aimed at understanding mechanisms of electric field-induced DNA transfection (17, 18, 25, 26). There are several crucial questions that continue to confound investigators. First, DNA used in these experiments have high molecular weights in the range of several millions. How can such large molecules enter a cell without eliciting some leakage of cytoplasmic macromolecules? Second, are there different pathways in which DNA transfer can be accomplished when PEF of different characteristics are employed? Third, how would the topology of DNA affect the transfection? Are differences in transfection efficiency (TE) a result of DNA entry, integration, or expression? Our previous study has shown that the TE of electric transfection correlated with the amount of DNA bound to the cell surface, implying that surface binding is required for the PEF-induced DNA entry (17). Furthermore, DNA added in the absence of an electric field could also transfect, implying that electrophoresis of DNA, while possible, is not a prerequisite for transfection (17). It was also shown that an ac field of amplitude one-tenth that is

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required for electroporation of cell membranes can also promote DNA transfection, suggesting the existence of alternative pathways for DNA entry (18). This study attempts to clarify factors affecting transfection efficiency. Further study will examine how DNA of different topologies may affect the transfection efficiency by the PEF method.

## MATERIALS AND METHODS

### Instruments

The set up for electric DNA transfection has been described previously (17–20). Basically, it consists of a high voltage generator (model 605P; Cober Electronics, Stamford, CT), and a cylindrical sample chamber with two platinized platinum electrodes in contact with the cell suspension. The two electrodes sit on two hollow stainless steel blocks, through which cooling water circulates to control the chamber's and hence the sample's temperature. The distance between the electrodes is adjustable between 0.1 and 0.5 cm, and the volume of the sample is 30–500 μl. In most cases, an electrode distance of 0.15 cm and a sample volume of 50 μl were used. The high voltage generator can deliver PEF of up to 2.2 kV, in different waveforms, with a width of 100 ns to 10 ms, either in single pulse or in sequence, with off duty duration of 100 ms or longer between two pulses. This study used only PEF of the rectangular waveform. The rise time of the electric pulse was ~30 ns. The waveform and the amplitude of a PEF across the two electrodes were directly monitored and displayed on a storage oscilloscope (model 5103; Tektronix, Beaverton, OR). The initial temperature of the sample was 3–5°C. After an electric pulse, the temperature could increase transiently, but it never exceeded 5°C. The temperature returned to the initial value in <2 s, as was monitored by a microthermistor probe with a time constant of 0.1 s. When multiple pulses were used, the time interval between pulses was 3 s.

### Preparation of cells and plasmid DNA

*E. coli* strain JM105 was grown in Luria Bertani (LB) medium, which contained 10 g bacto-tryptone, 5 g bacto-yeast extract, and 5 g NaCl per liter, with vigorous shaking at 37°C. The overnight culture grown to stationary phase was diluted 100 times into LB medium and grown to mid-log phase (specific optical density at 600 nm of 0.7–0.9, or cell density of $2–8 \times 10^8$ ml$^{-1}$) under normal conditions. For investigating effects of growth phase on transfection efficiency, cell suspensions with optical density between 0.3 and 4.5 (cell density ~ $5 \times 10^9$ ml$^{-1}$) were used for experiments. The cells were harvested and centrifuged at 400 *g* for 10 min at 4°C and washed twice with an appropriate electroporation medium (EM medium), which contained 2.5 mM $MgCl_2$, 30 mM sucrose, 1 mM Tris-HCl, pH 7.4. Cells were resuspended in the EM medium at a concentration of $1–2 \times 10^{10}$ ml$^{-1}$ and kept on ice before use.

Plasmid PUC$^{18}$, PUC$^{19}$, PBR$^{322}$, and PMSG were obtained from Pharmacia LKB Biotechnology, Inc. (Piscataway, NJ). They were diluted to an appropriate concentration in a medium (TE medium), which contained 10 mM Tris-HCl and 1 mM EDTA.

### Electroporation and assay for transfection efficiency

An appropriate amount of DNA stock solution was mixed with a cell suspension to give a final DNA concentration of 0.1 ng to 10 μg/ml and a cell concentration of $10^6$ to $10^{11}$ ml$^{-1}$ and was vortexed for 15 s. The mixture was incubated on ice for 5 min. 50 μl of cell–DNA mixture was transferred into the electroporation chamber, which was maintained at 4°C, and treated with 1–10 pulses of unipolar PEF of rectangular waveform, of amplitude up to 12 kV/cm and of duration between 30 μs and 10 ms. After the PEF treatment, the sample was immediately transferred to a 1.5-ml test tube that contained 500 μl of LB medium and incubated at 37°C for 30 min. After dilution by 10- to 10,000-fold, appropriate aliquots, usually 100 μl each, were subsequently plated in solid culture medium. The solid culture contained 15 g agar, 50 mg ampicillin, and 25 mg streptomycin per liter in the LB medium. When cell survival was to be examined, ampicillin was omitted from the culture. After overnight incubation at 37°C, colonies were counted. The TE was defined as the number of transformants, or colonies, per microgram DNA. The corrected transfection efficiency (CTE) was defined as the transfection efficiency divided by the percentage of cell survival.

## RESULTS

### Dependence of transfection efficiency on electric parameters

Data in Fig. 1 *A* show the transfection of *E. coli* treated with a single PEF of varied intensities and durations by the plasmid PBR$^{322}$. Log [TE] and percent cell survival are plotted versus field strength for PEF of the durations 40 μs, 200 μs, 1 ms, and 5 ms. The highest TE was obtained with PEF of 5-ms duration (TE of $3 \times 10^8$ μg$^{-1}$ DNA at 4 kV/cm), although in this case cell survival waned abruptly above 4 kV/cm. Cell survival was nearly 100% for PEF for shorter than 200 μs. This result was confirmed by a similar experiment in which transfection was done by varying the pulse width of the PEF (Fig. 1 *B*). Cell survival was nearly 100% for PEF of <200 μs up to 6 kV/cm. Log [TE] versus log (pulse width) was linear for pulse width of shorter than 1 ms for the intensity up to 6 kV/cm. Multiple application of PEF increased the TE. Log [TE] versus pulse number was linear up to 4 kV/cm when the pulse width was 1 ms (Fig. 1 *C*). Fig. 1 *D* shows a similar experiment done with varying field strengths.

### Effect of osmolality

The osmolality of the suspending medium appeared to have a pronounced effect on the efficiency of PEF-induced transfection of *E. coli* only when $Mg^{2+}$ was absent. The experiment shown in Fig. 2 used a 1-ms PEF of 8 kV/cm. Sucrose was used to adjust the osmolality of the suspending medium. Under these conditions, the maximal TE was obtained at 50 mM sucrose. Cell survival (75%) was unaffected in the entire range of sucrose concentrations used (1–300 mM). The TE at 50 mM was $10^7$ μg$^{-1}$ DNA, approximately fivefold greater than the TE at 300 mM sucrose. In the presence of 2.5 mM $Mg^{2+}$, TE was much higher, $5 \times 10^8$ μg$^{-1}$ DNA, as expected (17), but it was insensitive to the change in the osmolality of the suspending medium (data not shown).

### Dependence on types of plasmid DNA

PEF-induced transfection was more effective for PUC$^{18}$ and PUC$^{19}$, less effective for PBR$^{322}$, and least effective for PMSG. Fig. 3 compares TE of these plasmid DNAs

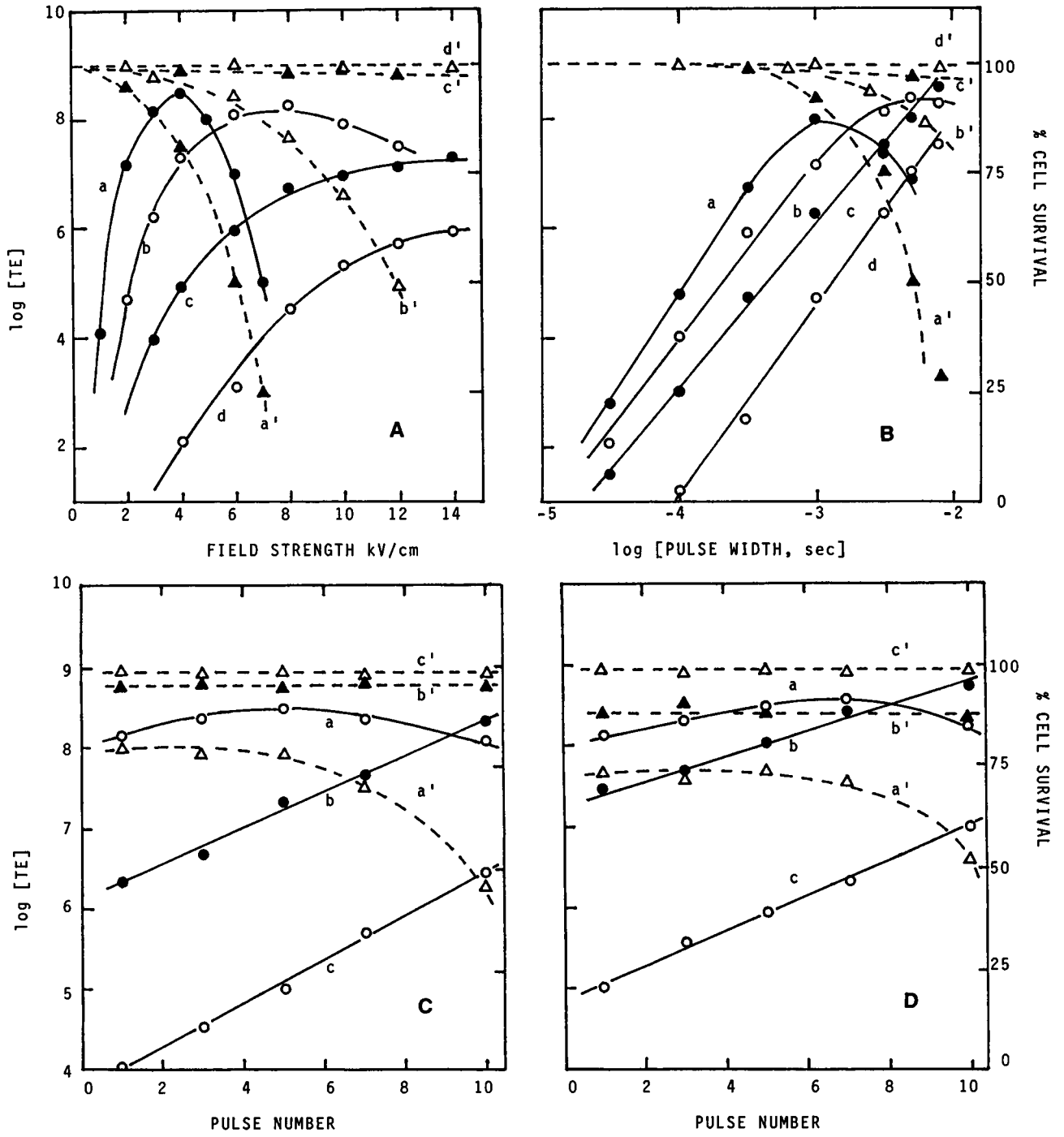


FIGURE 1 Effects of electric parameters on pBR$^{322}$ transfection of *E. coli* JM105. (*A*) Dependence of log [TE] on the field strength of PEF. The concentration of cells in the sample was $1.2 \times 10^{10}$ ml$^{-1}$, and DNA was 10 ng/ml. The electroporation medium contained 2.5 mM $MgCl_2$, 30 mM sucrose, 1 mM Tris-HCl buffer, pH 7.4. The temperature of the sample was maintained at 4°C. After the application of a single rectangular PEF, the temperature increased transiently (~1 s), but the increase never exceeded 5°C. The pulse durations were 5 ms, 1 ms, 200 μs, and 40 μs for curves *a*, *b*, *c*, and *d*, respectively. The corresponding curves (– – –) for percent cell survival are given in curves *a'*, *b'*, *c'*, and *d'*. The left ordinate indicates logarithm of the transfection efficiency and the right ordinate the percent cell survival. (*B*) Dependence of log [TE] on pulse width of PEF. The field strengths were 6, 4, 3, and 2 kV/cm, respectively, for curves *a*, *b*, *c*, and *d*. The corresponding curves (– – –) for percent cell survival are given in curves *a'*, *b'*, *c'*, and *d'*. Experimental conditions were identical to *A*. (*C*) Dependence of log [TE] on the number of electric pulses (3 kV/cm) of different durations. The durations for curves *a*, *b*, and *c* were 5 ms, 1 ms, and 200 μs, respectively. The corresponding curves (– – –) for percent cell survival are given in *a'*, *b'*, and *c'*. (*D*) Dependence of log [TE] on number of electric pulses (1-ms duration) of different strength. The strengths for curve *a*, *b*, and *c* were 6, 4, and 2 kV/cm, respectively. The corresponding cell survival curves are given in *a'*, *b'*, and *c'*.

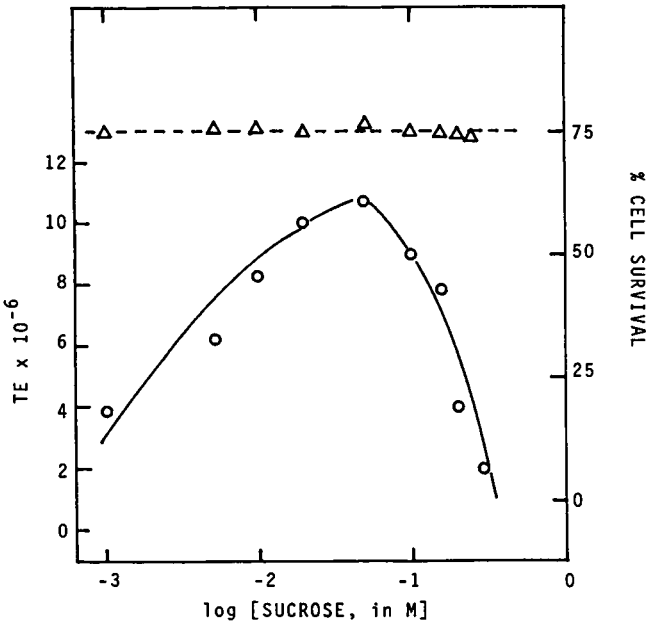


FIGURE 2 Effect of osmotic pressure of medium on transfection efficiency. Materials and experimental conditions used were identical to those specified in Fig. 1, except that here the media contained 1–300 mM, instead of 30 mM, of sucrose and $MgCl_2$ was omitted. A single 8-kV/cm PEF of 1-ms duration was applied to each sample. The percent cell survival is given in the broken line. In the presence of 2.5 mM of $MgCl_2$, the TE was much higher ($5 \times 10^8$ $\mu g^{-1}$ DNA) and was not altered significantly by the change in the osmolality of the medium.

under an identical set of electric parameters and solvent conditions. PUC[18] and PUC[19] DNAs are slightly different in sequences but with an identical molecular weight (2,686 base pair [bp], $1.6 \times 10^6$ mol wt). The TE was identical for these two plasmids. TE was nearly one order of magnitude greater for PUC[18] and PUC[19] DNA than that for PMSG (7626 bp, $4.7 \times 10^6$ mol wt) DNA. Intermediate TE was obtained for PBR[322] (4,362 bp, $2.2 \times 10^6$ mol wt). The likely cause of these differences in TE will be discussed.

## Dependence on growth phase of *E. coli*

Cells at different phases of growth responded differently to PEF treatment. Fig. 4 *A* shows that TE reached a maximal level when cells in the mid-log phase of growth were used for experiment. Generally, the mid-log phase was reached in ~2 h, and by 4–6 h the cells reached the stationary phase. TE declined by more than one order of magnitude for cells in the stationary phase. Cell resistance to PEF treatment followed the opposite trend of the TE curves, i.e., cells in the mid-log phase were most susceptible to PEF and became more resistant to PEF in the stationary phase. In this experiment, a single PEF of 8 kV/cm and of 1-ms duration was used. Fig. 4 *B* examines effects of field strength on cells harvested at different phases of growth. As is shown, the TE for cells in the mid-log phase of growth was 2.5 orders of magnitude greater than that of cells grown overnight.

## Other experimental parameters

For a given cell concentration, $10^{10}$ $ml^{-1}$, the number of cells transfected increased linearly with DNA concentration (Fig. 5 *A, open circles*). This being the case, the transfection efficiency, expressed as number of transformant per microgram of DNA, remained relatively constant for the DNA concentrations in the range 0.1 ng/ml to 10 $\mu$g/ml (Fig. 5 *A, closed circles*). Contrary to this observation, when DNA concentration was kept at 10 ng/ml, the transfection efficiency increased proportionately with increasing cell concentration (Fig. 5 *B*).

Temperature can increase lipid fluidity and facilitate resealing of electropores (2, 3). Its effect on transfection efficiency is shown in Fig. 6. TE decreased dramatically as the temperature for electroporation increased. Although the cell survival rate was also reduced, the decline in TE cannot be accounted for by the decrease in cell survival. The CTE showed a similar tendency to that of the TE curve. CTE decreased more than three orders of

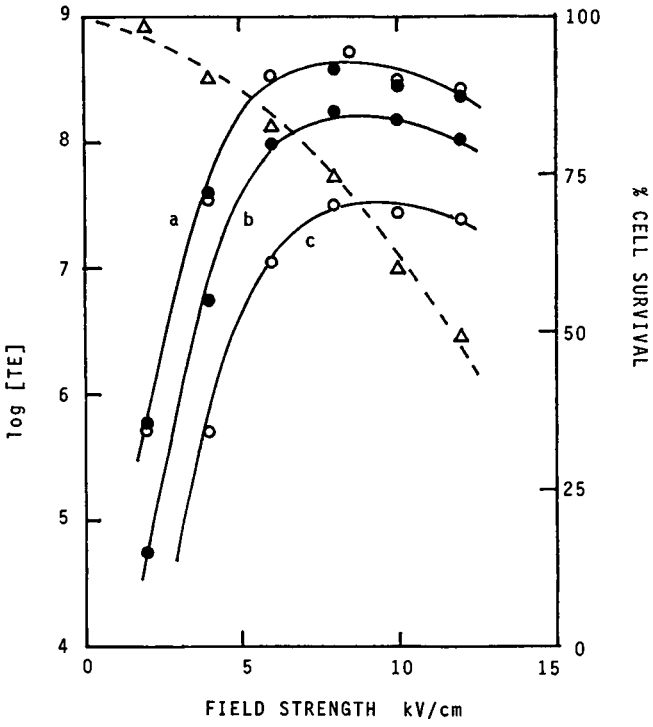


FIGURE 3 PEF-induced transfection of *E. coli* JM105 by different plasmids. The medium composition was identical to that of Fig. 1. The concentration of cell was $2 \times 10^{10}$ $ml^{-1}$ and of DNA was 10 ng/ml for PUC[18] and PUC[19], 16 ng/ml for PBR[322], and 28 ng/m for PMSG. This maintained the DNA/cell ratio at a constant value of 0.18. A single rectangular PEF of 1-ms duration and of varied field strength was used. Curve *a* was for the PUC[18] DNA (O) and PUC[19] DNA (●). Curves *b* and *c* were for the pBR[322] DNA and PMSG DNA, respectively. The cell survival curve is represented by the broken line.

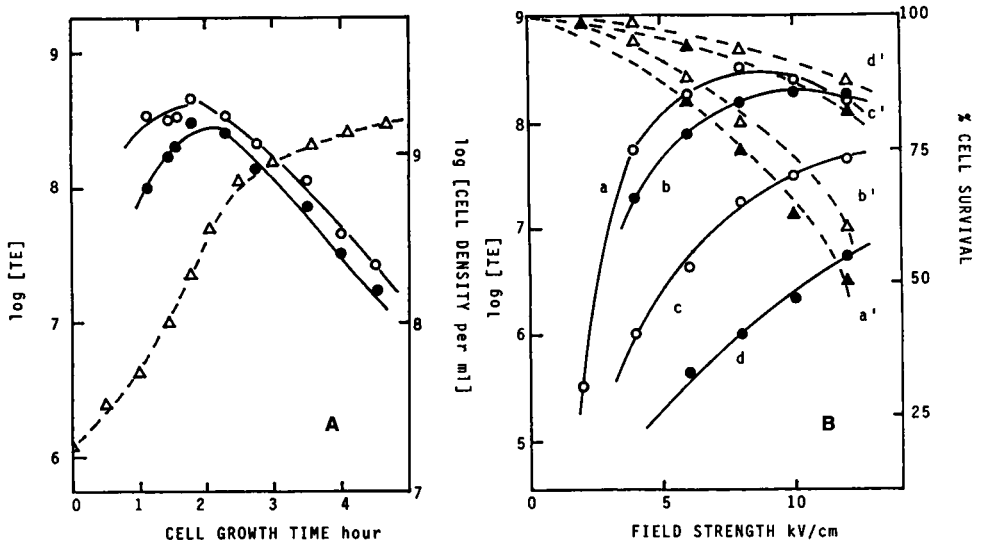


FIGURE 4 Transfection efficiency for cells harvested at different phases of cell growth. (*A*) Effects of growth phase. Cells harvested at different times after inoculation were treated with a single 8-kV/cm to 1-ms PEF. Data in the broken line show the cell density in the liquid culture. Logarithm of TE (●) and of CTE (○) were plotted versus the growth time. Other experimental conditions were identical to those of Fig. 1. (*B*) Dependence on field strength. Cells harvested at 1.5 h (curve *a*, culture cell density $2 \times 10^8$ ml$^{-1}$), 2.5 h (curve *b*, cell density $8 \times 10^8$ ml$^{-1}$), 4 h (curve *c*, cell density $3.2 \times 10^9$ ml$^{-1}$), and 14 h (curve *d*, cell density $3.2 \times 10^9$ ml$^{-1}$) were treated with a single PEF of 1-ms duration with varied field strength. The corresponding curves for cell survival are given in *a'*, *b'*, *c'*, and *d'*.

magnitude when the temperature increased from near 0 to 37°C.

Another important factor that can influence transfection efficiency is the pH. Data in Fig. 7 *A* indicate (not surprisingly) that PEF treatment at neutral pH is best for both cell survival and DNA transfection. Again, the variation in TE cannot be accounted for by cell viability alone. The CTE curve showed a similar shape to that of the TE curve. Fig. 7 *B* gives experimental data obtained by changing field strength for different pH values.

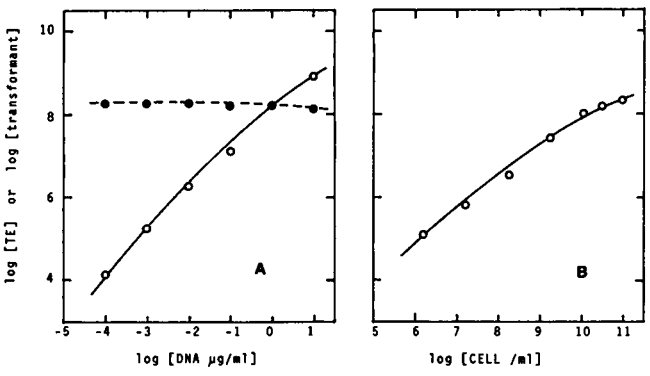


FIGURE 5 Effects of DNA and cell concentrations on number of cells transfected and on log [TE]. (*A*) Dependence on DNA (pBR$^{322}$) concentration. The experimental conditions were identical to those of Fig. 1, except that here a single 8-kV/cm to 1-ms PEF was applied to each sample. Curve shown in open circles plots logarithm of number of transformants produced versus log [DNA], and curve shown in filled circles plots log [TE] versus log [DNA]. (*B*) Dependence on cell number. DNA (pBR$^{322}$) concentration was 10 ng ml$^{-1}$. A single 8-kV/cm to 1-ms PEF was applied to each sample.

## DISCUSSION

Stronger electric field and longer pulse duration are known to implant pores of larger sizes in cell membranes, which in turn can enhance DNA entry and improve the transfection efficiency. However, these conditions can also cause irreversible damage to cell membranes and consequently greater cell death (1, 2). The accurate assessment of transfection efficiency is to correct for the cell death represented in this study as corrected transfection efficiency (CTE). The present study indicates that there are optimal combinations of electric parameters and solvent conditions for achieving high CTE. With the limited conditions used in our experiments, we have recorded a TE of $10^{10}$ per μg DNA. The conditions used were 10 ng ml$^{-1}$ PUC$^{18}$ DNA, $1.2 \times 10^{10}$ ml$^{-1}$ cell, 2.5 mM $MgCl_2$, 50 mM sucrose, 1 mM Tris buffer, pH 7.4, and one 8-ms PEF of 4 kV/cm. Because, under the conditions used, only 40–50% of DNA would be bound to the cell surface, i.e., roughly $1.5 \times 10^{11}$ copies of surface bound DNA (17), a TE of $10^{10}$ per μg DNA would mean that ~7% of cells with a copy or more of DNA bound to their surface resulted in transfection. The CTE would be ~10%. At very high DNA/cell ratios, transfection of as much as 50% of the cells has been reported by Zheng and Chang (27) for cells in the monolayer culture.

Our data show that to achieve greater yield of transformants, a higher concentration of DNA and cells may be used. Fig. 5 *A* shows that a nearly constant TE of $1.5 \times 10^8$ per μg DNA was obtained for DNA concentration of 0.1 ng to 10 μg/ml. Thus, in practice, if DNA is scarce

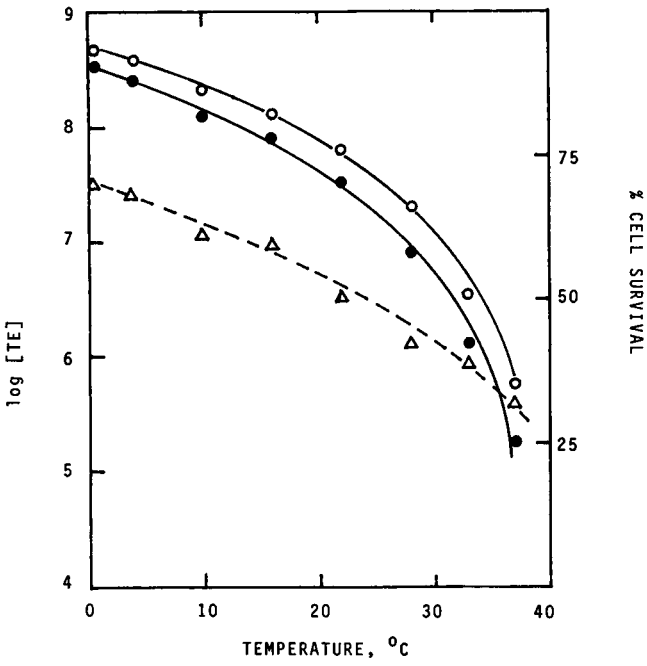


FIGURE 6 Effects of temperature on transfection efficiency. Cell concentration was $1.5 \times 10^{10}$ and the DNA ($PUC^{18}$) concentration was 10 ng/ml. A single 8-kV/cm to 1-ms PEF was used. Other conditions were identical to those of Fig. 1. Data in the filled circles are for the transfection efficiency and those in the open circles are for the corrected transfection efficiency (CTE). Curve for the cell survival is represented by the broken line.

and cells are abundant, a low DNA/cell ratio may be selected to achieve the maximal economy. Conversely, if DNA is abundant and cells are precious, a high DNA/cell ratio should be chosen. Because a high DNA/cell ratio did not improve substantially the fraction of cells transfected, one may conclude that not every cell in a sample was transfection competent. This is consistent with the observation that the TE for cells grown overnight was 2.5 orders of magnitude lower than that for cells in the mid-log phase of growth.

The differences in TE obtained for different plasmid DNAs cannot be attributed to the differences in the DNA/cell ratios because, as shown in Fig. 5 *A*, TE was nearly constant for the wide range of DNA/cell ratios used. There are at least four factors that can influence DNA entry into a cell: the electrophoretic driving force (26), the electroosmosis (28, 29), the diffusion rate, and the average pore size. DNA electrophoretic mobility is known to be independent of the number of base pairs. Thus, the TE should not differ for DNA of different sizes. Data in Fig. 3 show that this was not the case. Therefore, a direct link between TE and the electrophoretic driving force cannot be established for the present system. Electroosmosis will generate a flow of water toward the cathode (28, 29). DNA in the vicinity of a cell may be carried into the cell by this hydrodynamic flow. Because the speed of the flow is unlikely to be affected by the size of the DNA present in the medium, TE should not depend dramatically on the size of the DNA.

Diffusion of a smaller DNA is expected to be faster than that of a larger DNA. If diffusion of surface-bound DNA through electropores is the mechanism, TE will be greater for a small DNA compared with that for a large DNA. The diffusion coefficient for a spherical particle is $D = RT/(N_0 f)$, where $R$ and $N_0$ are, respectively, the gas constant and the Avogadro's number. The frictional coefficient is $f = 6\pi\eta r$, where $\eta$ and $r$ are, respectively, the viscosity of the medium and the radius of the particle. Since $r = (3v/4\pi)^{1/3} M^{1/3}$, where $v$ is the partial specific volume of DNA in a dilute salt solution (roughly 0.7

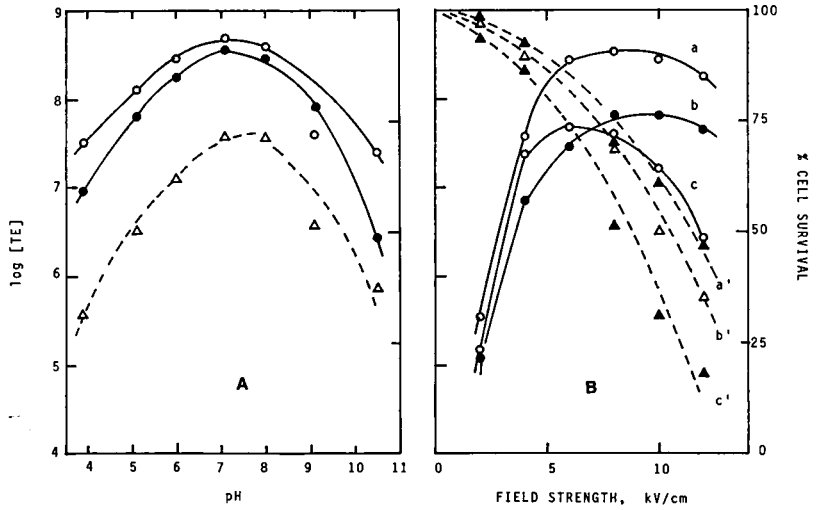


FIGURE 7 Effects of pH on transfection. (*A*) pH dependence. DNA, cell, and experimental conditions were identical to those given in Fig. 6. Electroporation was performed at a given pH, and logarithms of TE (●) and CTE (○) are plotted versus pH. Cell survival is represented by the broken line. (*B*) Dependence of log [TE] on field strength at different pH. A single PEF of 1-ms duration with varied field strength at pH 7.15 (curve *a*), 5.12 (curve *b*), and 9.05 (curve *c*). The corresponding curves for cell survival are *a'*, *b'*, and *c'*.

g/ml) and $M$ is the molecular weight, $D$ should be inversely proportional to $M^{1/3}$. As can be seen from the results in Fig. 3, TE showed a more pronounced dependence on the molecular weight of DNA. The TE was roughly inversely proportional to $M^2$, i.e., a threefold increase in the size of DNA resulted in the reduction of TE by eightfold. One should recognize that *E. coli* has a peptidoglycan/lipopolysaccharide cell wall. And the plasmid DNA are by no means spherical in shape. A recent study of the diffusion of flexible polymers at the solid intersurface indicates that the diffusion coefficient is inversely proportional to the square of the polymer molecular weight (30). This being the case, it is likely that all four factors mentioned can exert influences on transfection efficiency. Quantitative analysis of these results remains to be done.

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## REFERENCES

1. Neumann, E., A. E. Sowers, and C. A. Jordan, editors. 1989. Electroporation and Electrofusion in Cell Biology. Plenum Press, New York. 436 pp.
2. Tsong, T. Y. 1991. Electroporation of cell membranes. *Biophys. J.* 60:297–306.
3. Kinosita, K., Jr., and T. Y. Tsong. 1977. Formation and resealing of pores of controlled sizes in human erythrocyte membrane. *Nature (Lond.).* 268:438–441.
4. Auer, D., G. Brandner, and W. Bodemer. 1976. Dielectric breakdown of the red blood cell membrane and uptake of SV40 DNA and mammalian cell RNA. *Naturwissenschaften.* 63:391.
5. Wong, T.-K., and E. Neumann. 1982. Electric field mediated gene transfer. *Biochem. Biophys. Res. Commun.* 107:584–587.
6. Neumann, E., M. Schaefer-Ridder, Y. Wang, and P. H. Hofschneider. 1982. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:841–845.
7. Potter, H., L. Weir, and P. Leder. 1984. Enhancer-dependent expression of human $\kappa$ immunoglobulin genes introduced into pre-B lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA.* 81:7161–7165.
8. Smithies, O., R. G. Gregg, S. S. Boggs, M. A. Koralewski, and R. S. Kucherlapati. 1985. Insertion of DNA sequences into the human chromosomal $\beta$-globin locus by homologous recombination. *Nature (Lond.).* 317:230–233.
9. Chu, G. H., H. Hayakawa, and P. Berg. 1987. Electroporation for efficient transfection of mammalian cells with DNA. *Nucleic Acids Res.* 15:1311–1326.
10. Fromm, M. E., L. P. Tayloer, and V. Walbot. 1986. Stable transfection of maize after gene transfer by electroporation. *Nature (Lond.).* 319:791–793.
11. Ou-Lee, T. M., R. Turgeon, and R. Wu. 1986. Expression of a foreign gene linked to either a plant-virus or a Drosophila promoter, after electroporation of protoplasts of rice, wheat and sorghum. *Proc. Natl. Acad. Sci. USA.* 83:6815–6819.
12. Saunders, J. A., B. F. Matthews, and P. D. Miller. 1989. Plant gene transfer using electrofusion and electroporation. *In* Electroporation and Electrofusion in Cell Biology. E. Neumann, A. E. Sowers, and C. A. Jordan, editors. Plenum Press, New York. 343–354.
13. Karube, I., E. Tamiya, and H. Matsuoka. 1985. Transformation of *Saccharomyces cerevisiae* spheroplasts by high electric pulse. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 205:179–184.
14. Colvin, N. M., and P. C. Hanawalt. 1988. High-efficiency transfection of bacteria by electroporation. *J. Bacteriol.* 170:2796–2801.
15. Dower, W. J., J. F. Miller, and C. W. Ragsdale. 1988. High-efficiency transformation of *E. coli* by high voltage electroporation. *Nucleic Acids Res.* 16:6127–6145.
16. Fiedler, S., and R. Wirth. 1988. Transformation of bacteria with plasmid DNA by electroporation. *Anal. Biochem.* 170:38–44.
17. Xie, T. D., L. Sun, and T. Y. Tsong. 1990. Study of mechanisms of electric field induced DNA transfection. I. DNA entry by binding and diffusion through membrane pores. *Biophys. J.* 58:13–19.
18. Xie, T. D., and T. Y. Tsong. 1990. Study of mechanisms of electric field induced DNA transfection. II. Transfection by low amplitude, low frequency alternating electric fields. *Biophys. J.* 58:897–903.
19. Kinosita, K., Jr., and T. Y. Tsong. 1977. Hemolysis of human erythrocytes by transient electric field. *Proc. Natl. Acad. Sci. USA.* 74:1923–1927.
20. Serpersu, E. H., K. Kinosita, Jr., and T. Y. Tsong. 1984. Reversible and irreversible modification of erythrocyte membrane permeability by electric field. *Biochim. Biophys. Acta.* 812:779–785.
21. Forster, W., and E. Neumann. 1989. Gene transfer by electroporation: a practical guide. *In* Electroporation and Electrofusion in Cell Biology. E. Neumann, A. E. Sowers, and C. A. Jordan, editors. Plenum Press, New York. 299–318.
22. Potter, H. 1988. Electroporation in biology: methods, applications, and instrumentation. *Anal. Biochem.* 174:361–373.
23. Chang, D. C. 1989. Cell poration and cell fusion using an oscillating electric field. *Biophys. J.* 56:1053–1058.
24. Tekle, E., R. D. Astumian, and P. B. Chock. 1991. Electroporation by using bipolar oscillating electric field: an improved method for DNA transfection of NIH 3T3 cells. *Proc. Natl. Acad. Sci. USA.* 88:4230–4234.
25. Chernomordik, L. A., A. V. Sokolov, and V. G. Budker. 1990. Electrostimulated uptake of DNA by liposomes. *Biochim. Biophys. Acta.* 1024:179–183.
26. Klenchin, V. A., S. I. Sukharev, S. M. Serov, L. V. Chernomordik, and Y. A. Chizmadzhev. 1991. Electrically induced DNA uptake by cells is a fast process involving DNA electrophoresis. *Biophys. J.* 60:804–811.
27. Zheng, Q., and D. C. Chang. 1990. High-efficiency gene transfection of attached mammalian cells by a radio-frequency electric field. *Biochim. Biophys. Acta.* 1088:104–110.
28. Sowers, A. E. 1989. The mechanism of electroporation and electrofusion in erythrocyte membranes. *In* Electroporation and Electrofusion in Cell Biology. E. Neumann, A. E. Sowers, and C. A. Jordan, editors. Plenum Press, New York. 229–256.
29. Dimitrov, D. S., and A. E. Sowers. 1990. Membrane electroporation—fast molecular exchange by electroosmosis. *Biochim. Biophys. Acta.* 1022:381–392.
30. Whitlow, S. J., and R. P. Wool. 1991. Diffusion of polymers at interfaces: a secondary ion mass spectroscopy study. *Macromolecules.* 24:5926–5938.