J. Cell Sci. 49, 87–97 (1981)

QUANTITATIVE CELL FUSION: THE FUSION SENSITIVITY (FS) POTENTIAL

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SUMMARY

The dose response of Sendai virus-induced cell fusion was studied in 10 mammalian cell lines, comprising 5 continuous and 5 diploid cell lines originating from 5 species. The extent of fusion was calculated using a parameter directly proportional to the number of fusion events (t-parameter). At lower levels of fusion the dose response was found to be based on the same simple kinetic rules in all cell lines and was defined by the formula: $t = FS \cdot FAU/(1 + FS \cdot FAU)$, where FS (fusion sensitivity) is a cell-specific constant of the fusion rate and FAU (fusion activity units) is the virus dose. The FS potential of a cell line was determined as the linear regression coefficient of the fusion index ($t/(1-t)$) on the virus dose. At higher levels of fusion, when the fusion extent reached cell-line-specific maximal levels, the dose response was not as uniform. In general, and particularly in the cases of the diploid cell lines, these maximal levels were directly proportional to the FS potentials. Thus, it was concluded that the FS potential is the basic quantitative feature, which expresses the cellular fusion efficiency. The fact that FS varied extensively between cell lines, but at the same time apparently followed certain patterns (being higher in continuous compared to diploid cell lines and being related to the species of origin of the cells), emphasizes its biological significance as well as its possible usefulness in studies of the efficiency of various molecular interactions in the cell membrane/cytoskeleton system.

INTRODUCTION

Inactivated Sendai virus, which is the most commonly used experimental fusing agent, has the ability to induce cell fusion in all vertebrate cells (Harris, 1970), although to a markedly different degree in different cell types (Harris, 1970; Okada, 1969; Poste & Pasternak, 1978; Graves & Hope, 1977). Evidence from many reports indicates that, in general, fusion sensitivity is more pronounced in continuous cell lines and tumour cells than in primary cell cultures or diploid cell lines (Okada & Tadokoro, 1963; Poste, 1970; Graves & Hope, 1977).

Although cell fusion *in vivo* is restricted to a few cell types (see Ringertz & Savage, 1976), cell-membrane fusion is involved in a number of secretory and excretory processes of cells (Orci & Perrelet, 1978; Chi, Lagunoff & Koehler, 1976). It is thus reasonable to assume that the different rates at which cells participate in experimentally induced cell fusion may be an expression either of their potential rate of performing cell-membrane fusions (particularly if regarding cell fusion as an exaggeration of cell-membrane fusion processes) or of some other general potential of the cell membrane and/or the cytoskeleton.

In a previous study that dealt with the validity of certain theoretical cell fusion models of multinuclearity (Röhme & Thorburn, 1981), it was concluded that all cells in a cell line participate in fusion with about the same probability. Thus, differences in

fusion sensitivity between cell lines are not likely to be due to variation in the proportion of cells capable of fusion, but are due rather to quantitative differences expressed at the cellular level. In this investigation efforts are made to find a simple quantitative means for an accurate estimation of the cellular response to the fusing effect of Sendai virus. It is demonstrated that, under standard conditions, this response could be expressed as linear to the virus dose and is the function of a cell-line-specific constant of the fusion rate, the fusion sensitivity (FS) potential.

MATERIALS AND METHODS

Cell lines

A total of 10 mammalian cell lines derived from 5 different species were used (Table 1). Five of these cell lines were diploid fibroblasts (F2000, HES, RL, RES and ME) and the other 5 were continuous cell lines derived from tumours of normal tissues which were 'spontaneously' altered *in vitro* (Lu106, HeLa S_3, D98-AH, ELD and PTO). These continuous cell lines were all heteroploid and epithelial-like in appearance. The diploid fibroblasts were used when in passage 8 to 15 in the case of F2000 and HES, and in passage 2 to 5 in the case of RL, RES and ME.

Table 1. *Origin and classification of the* 10 *cell lines used*

Cell line	Origin	Type of cell culture*	Reference
HeLa S_3	Human, cervix carcinoma	Continuous	Flow laboratory
Lu106	Human, probably a HeLa derivative	Continuous	Heneen (1976)
D98-AH	Human, probably a HeLa derivative	Continuous	Heneen (1976)
F2000	Human, embryonic lung	Diploid	Flow laboratory
HES	Human, embryonic skin	Diploid	This laboratory
ELD	Mouse, Ehrlich ascites tumour	Continuous	Nielsén (1972)
ME	Mouse, whole embryo	Diploid	This laboratory
RES	Rat, embryonic skin	Diploid	This laboratory
RL	Rabbit, adult lung	Diploid	This laboratory
PTO	Rat kangaroo, ovary	Continuous	This laboratory

* Terminology according to the recommendation of the Tissue Culture Association (Schaeffer, 1979).

All cell lines were grown as monolayer cultures in Eagle's medium supplemented with 15 % foetal calf serum (FCS), 100 μg/ml streptomycin and 100 units/ml penicillin. HeLa S_3, normally grown in suspension culture, were grown as monolayers 2 serial passages prior to the fusion experiments.

Mycoplasma contamination has been monitored and found negative in F2000, HES, RL, RES and ME cells, and positive in Lu106 cells. The remaining cell lines have not been tested in this respect.

Cell fusion

Cell fusion was induced with inactivated Sendai virus in suspensions as previously described (Röhme & Thorburn, 1981). The cells were first harvested with trypsin and collected in Eagle's medium with 15 % FCS. After 5 min the cells were washed twice in the same medium without FCS, before a cold (4 °C) Sendai virus suspension was added. The final concentration was always 10^6 cells per 100 μl of total solution (usually 100–500 μl). After 15 min in the cold (4 °C), the test tubes were transferred to a warm (37 °C) water shake-bath. After 40 min the cells were diluted and gently aspirated in Eagle's mdium with 15% FCS and left for another

5 min in a 37 °C water shake-bath. The cells were then aspirated more heavily and diluted further in the same medium to ensure low cell densities when plated out in Petri dishes containing coverslips. Fused cells and controls were incubated at 37 °C in a 5% CO_2 atmosphere for about 18 h, which is enough time for the completion of the fusion process and the flattening out of cells (Röhme, unpublished data). The cells were then fixed in methanol for 5 min, stained in a Giemsa solution (1 part of Giemsa stock solution diluted with 9 parts H_2O) for 2–5 min, dehydrated in acetone and finally via 2 baths of xylene before being mounted in DePex. The number of nuclei per cell was counted microscopically in about 1000 cells.

Quantification of fusion

Study of the quantitative nature of cell fusion in relation to the virus dose is preferably done by relating the number of fusion events to the virus dose. This is possible when using the t-parameter as a measure of the extent of fusion, as recently introduced by Röhme & Thorburn (1981). This parameter can be calculated as

$$t = (\mathrm{ANC} - 1)/\mathrm{ANC},$$

where ANC is the average number of nuclei per cell. This formula is valid in an ideal cell population consisting of only mononucleate cells. The small percentage of polykaryocytes usually found in cell cultures can be corrected for as follows:

$$t = (\mathrm{ANC_E} - \mathrm{ANC_C})/\mathrm{ANC_E}. \quad (1)$$

E, experimental culture, C, control culture.
The ratio $t/(1-t)$ is termed the fusion index (FI) and can be calculated using formula (1) as

$$\mathrm{FI} = (\mathrm{ANC_E} - \mathrm{ANC_C})/\mathrm{ANC_C}. \quad (2)$$

Basically FI is the same parameter as that introduced by Okada & Tadokoro (1962). When $\mathrm{ANC_C}$ is 1·0, FI = ANC − 1, which is the parameter used by these investigators although they expressed it in another way.

Fusion activity units (FAU)

The titres (as to fusing capacity) of the virus preparations used are calculated in fusion activity units. One FAU is the virus dose fusing 10^6 HeLa S_3 cells per 100 μl solution to give a t value of 0·1 (or FI = 0·111). Using these cells, the FAU of a virus preparation is calculated as

$$\mathrm{FAU} = \mathrm{FI}/0{\cdot}111, \quad \text{when} \quad \mathrm{FI} < 0{\cdot}2 \quad (3)$$

or, using Lu106 cells as a standard reference line, the equivalent formula is

$$\mathrm{FAU} = \mathrm{FI}/0{\cdot}139, \quad \text{when} \quad \mathrm{FI} < 0{\cdot}2. \quad (3')$$

(For derivation, see Results.)

Fusion sensitivity (FS)

The FS potential is a cell-line-specific constant of the fusion rate in response to the virus dose as defined by the formula:

$$\mathrm{FS} = t/(1-t)\cdot 1/\mathrm{FAU}, \quad \text{when} \quad t < 0{\cdot}2. \quad (4)$$

Since $t/(1-t)$ = FI (definition), FS may simply be determined as

$$\mathrm{FS} = \mathrm{FI}/\mathrm{FAU}, \quad \text{when} \quad \mathrm{FI} < 0{\cdot}2. \quad (5)$$

(For derivation, see Results.)

RESULTS

Virus dose response and the fusion sensitivity potential

The extent of fusion of Lu106 cells obtained after treatment in suspension with a wide range of virus doses is shown in Fig. 1. This dose response is based on 2 experimental sets comprising 13 experiments. The extent of fusion is calculated and plotted

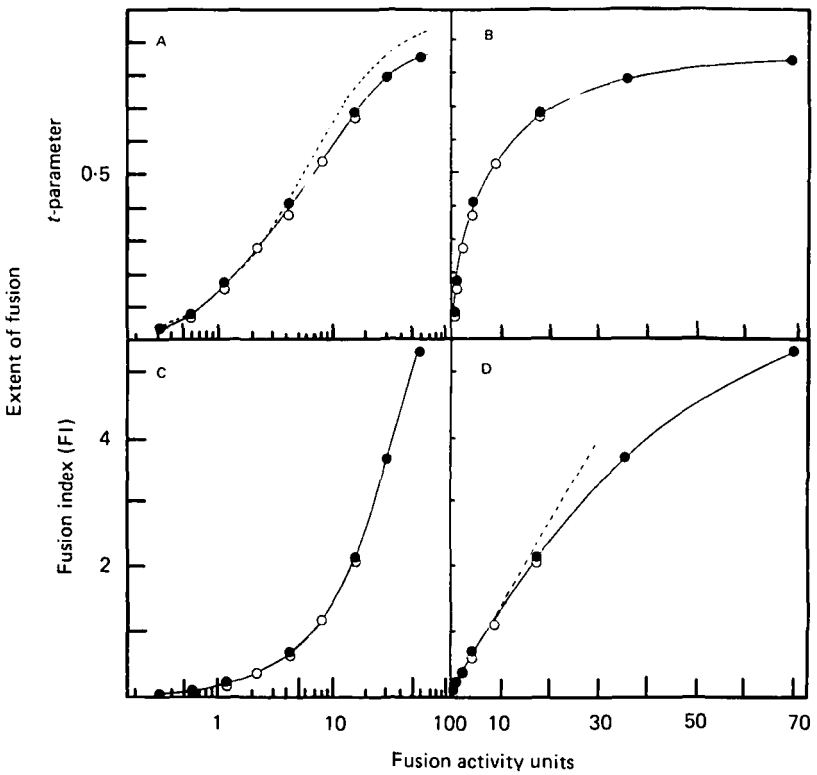

Fig. 1. Dose–response curves for fusions of Lu106 cells in suspensions expressed in different scales of the virus dose, (A) and (C) logarithmic, (B) and (D) linear. ——, observed, - - - -, expected from: (A) formula (6), and (D) formula (7) by using the FS values of Lu106 given in Table 2.

using both the t and the FI parameters in linear and logarithmic scales of the virus dose. The nature of the dose response is particularly elucidated by the type of plots shown in Fig. 1A (t as a function of the logarithm of the virus dose) and in Fig. 1D (FI as a function of the absolute virus dose). It appears from Fig. 1D that the FI is linearly related to the absolute virus dose for FI values up to about 2. This means that up to this fusion level FI = FS·FAU. Since by definition FI = $t/(1-t)$, the equivalent dose relation for t would be t = FS·FAU/(1 + FS·FAU). When plotting FAU on a logarithmic scale, t will be expressed by a typical sigmoid curve as shown in Fig. 1A. When using the FS value for Lu106 cells given in Table 2, 0·1387, it is apparent that the expected curve plotted in Fig. 1A fits the experimental data rather

nicely when $t < 0{\cdot}5$. Thus, in the case of Lu106, the virus dose response of fusion at lower levels of fusion may be expressed by the simple formulae

$$t = \mathrm{FS}\cdot\mathrm{FAU}/(1+\mathrm{FS}\cdot\mathrm{FAU}) \tag{6}$$

or

$$\mathrm{FI} = \mathrm{FS}\cdot\mathrm{FAU}. \tag{7}$$

Similar dose-response experiments were carried out in 9 other cell lines, comprising 4 continuous and 5 diploid cell lines (Fig. 2). It is evident that these cell lines demonstrate large differences as regards the fusion sensitivity (FS) and the maximal

Table 2. *The fusion sensitivity potential of* 10 *mammalian cell lines*

Cell line	FS potential* ($\times 10^2$)	Correlation coefficient†
Lu106	13·87	0·995
HeLa S3	11·11	0·991
D98	6·85	0·982
PTO	6·23	0·991
ELD	2·31	0·996
F2000	1·94	0·991
HES	1·66	0·990
RL	0·58	0·992
RES	0·46	0·991
ME	0·40	0·993

* Calculated as the linear regression coefficient of the fusion index (FI) on the virus dose (FAU/10^6 cells per 0·1 ml), when FI < 0·20 in all cell lines except RL, RES and ME, in which FI < 0·10.

† All values in this column are significant at $P < 0{\cdot}001$.

levels of fusion (MLF) attained. Firstly, it may be noticed that all 4 continuous cell lines fuse more readily than the diploid cell lines. As shown in Table 2, the FS potentials differ about 35-fold between the extreme cases, human Lu106 and mouse ME cells.

Secondly, there seems to be an overall correlation between the FS and the MLF obtained in a cell line (Figs. 1A, 2). In particular this correlation seems to be very good for all of the 5 diploid cell lines (Fig. 3), whereas continuous cell lines may vary in this respect. The very sensitive cell lines, Lu106 and HeLa S3, reach very high MLF values and the somewhat less sensitive PTO cells also reach a comparatively high MLF. However, the D98-AH cells, which are about as sensitive as PTO cells, do not reach a MLF value even as high as the considerably less sensitive human diploid fibroblasts, F2000 and HES. Also, in case of ELD cells the MLF is comparatively low. It should also be taken into account that cell lysis seems to increase somewhat in all cell lines as they reach MLF. Although not quantitatively estimated, the degree of cell lysis was much more pronounced in the cases of D98-AH and ELD.

Thirdly, the initial phases of all curves (Figs. 1A, 2) appear to be very similar, i.e.

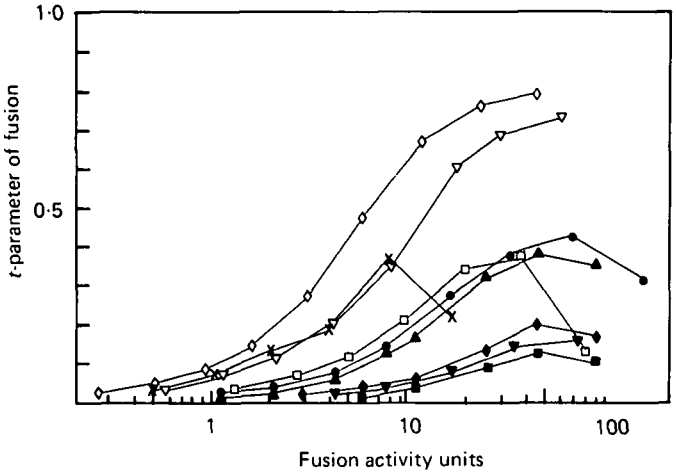

Fig. 2. Dose–response curves for fusions of 9 cell lines in suspension: HeLa S3 (human) (◇—◇); D98-AH (human) (×—×); PTO (rat kangaroo) (▽—▽); ELD (mouse) (□—□); F2000 (human) (●—●); HES (human) (▲—▲); RL (rabbit) (◆—◆); RES (rat) (▼—▼); ME (mouse) (■—■).

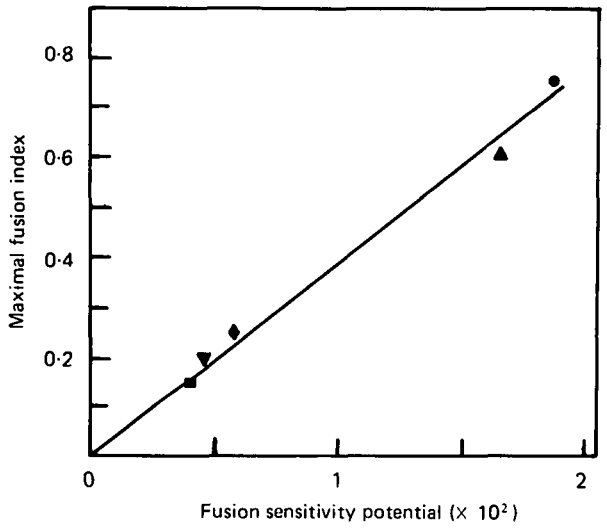

Fig. 3. The linear correlation between the fusion sensitivity potential of 5 diploid fibroblastic cell lines and the maximal level of fusion obtained in these cell lines: ●, F2000 (human); ▲, HES (human); ◆, RL (rabbit); ▼, RES (rat); ■, ME (mouse). The correlation coefficient, 0·993, is significant at $P < 0{\cdot}001$.

the relative increase in the virus dose needed to increase the extent of fusion from one level to another is the same. In fact, calculating the extent of fusion as FI, there is a very good linear relation to the virus dose, when FI = 0·1 in the case of rodent diploid cell lines and FI = 0·2 in the cases of the other cell line (Fig. 4 and Table 2). Thus the virus response at this comparatively low level of fusion in all 10 cell lines

may be characterized very precisely by the value of the FS. Accordingly, this cell-specific constant of the fusion rate is termed the FS potential, and is defined as

$$FS = t/(1-t)\cdot 1/FAU, \quad \text{when} \quad t < 0{\cdot}2 \tag{4}$$

and may be simply determined as

$$FS = FI/FAU, \quad \text{when} \quad FI < 0{\cdot}2. \tag{5}$$

To determine the FS of cells with very low sensitivity, like diploid rodent or rabbit cells, the levels of fusion should be $t < 0{\cdot}1$ or $FI < 0{\cdot}1$. This low level of fusion is thus preferable for FS determinations of all cell lines.

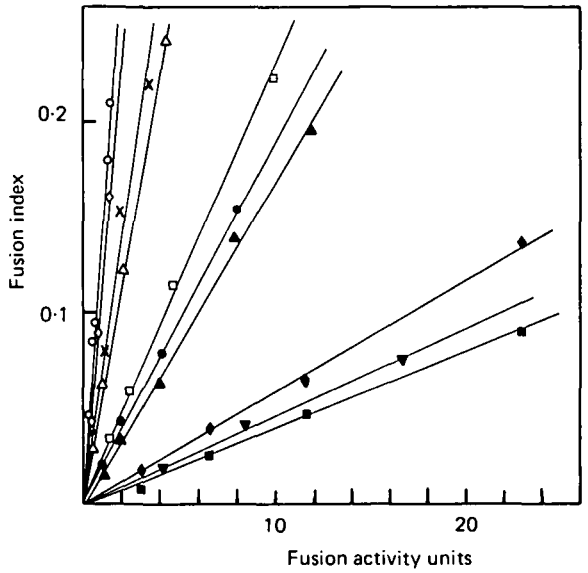

Fig. 4. Linear correlations between the extent of fusion and the virus titre at low levels of fusion obtained in suspensions of 10 cell lines: Lu106 (human) (○—○); HeLa S3 (human) (◇—◇); D98-AH (human) (×—×); PTO (rat kangaroo) (△—△); ELD (mouse) (□—□); F2000 (human) (●—●); HES (human) (▲—▲); RL (rabbit) (◆—◆); RES (rat) (▼—▼); ME (mouse) (■—■).

Fusion activity units (FAU)

A reliable estimate of the FS potential of a cell line obviously requires a correct estimate of the virus dose. Since the fusing activity of a virus preparation may vary after storage at −70 to −90 °C (Röhme, unpublished data), a simple standard means was introduced to determine this activity in each experiment. For this purpose, fusion experiments with a standard cell line, HeLa S3 or Lu106, were always carried out together with the other cell lines to be studied. Thus, one fusion activity unit was defined as the virus dose fusing a suspension of 10^6 HeLa S3 cells per 100 μl of suspension to an extent of $t = 0{\cdot}10$, according to the procedures given in Materials and methods. Using this definition of FAU, and formulae (4) and (5), the FS potential of HeLa S3 will be $FS = t(1-t)\cdot FAU = FI/FAU = 0{\cdot}111$. Using this FS value, the

FAU titre for a virus preparation may simply be calculated from a fusion experiment with HeLa S3 cells by formula (3) (see Materials and methods). Using Lu106 cells (FS = 0·139, Table 2) as a standard reference line, the equivalent formula is (3′) (see Materials and methods).

DISCUSSION

The dose response of Sendai virus-induced cell fusion was determined in 10 mammalian cell lines. It is concluded that at lower levels of fusion the dose response follows the same simple kinetic rules in all cell lines studied. Thus, when calculating the extent of fusion using the FI parameter, the fusion response was linear to the virus dose and the extent of fusion was a function of the cell-line-specific constant of the fusion rate, the fusion sensitivity (FS) potential. The differences between cell lines in their fusion response therefore could be accurately estimated by this FS potential.

As to the nature of the dose response, a linear relation has also been demonstrated by other investigators using the same FI parameter (Okada & Murayama, 1968; Okada, 1969). However, in contrast to the results obtained here, these investigators found that a threshold dose of virus was required before fusion was initiated. In most other studies concerning the dose response of virus-induced cell fusion, there was a linear relation of the type obtained here at lower levels of fusion, if the results were recalculated using the same FI parameter (Okada & Tadokoro, 1962, 1963; Kohn, 1965; Velasquez, Payne & Krooth, 1971; Wainberg, Howe & Godman, 1973). At higher levels of fusion the kinetics become more complicated, since the extent of fusion reaches cell-line-specific maximal levels. Particularly in the cases of diploid cell lines, these maximal levels of fusion were directly proportional to the FS potential. In continuous cell lines, on the other hand, there were deviations from this general trend. Thus, although we cannot presently express the overall kinetics of the dose response of cell fusion in a strict formula, the FS potential probably is the basic quantitative feature that expresses the fusion efficiency of a cell line.

As to the differences in the FS potentials of cell lines, they varied some 35-fold between the extremes, human Lu106 and mouse ME cells. There are 2 aspects to be commented upon in this connexion. Firstly, although the FS potentials of the 5 continuous cell lines varied up to 6-fold, they were all higher than those of the diploid cell lines. Such differences in fusion sensitivity between continuous and diploid cell lines are in agreement with the results of many other investigators (Harris, 1970; Okada & Tadokoro, 1963; Graves & Hope, 1977; Poste & Pasternak, 1978). Secondly, there appears to exist a relation between the FS and the species of origin of the cells. This was particularly evident in the case of diploid cell lines, which showed a difference in FS of up to about 5-fold. In relation to the species of origin of these cell lines, the FS potential decreased in the order: man, rabbit, rat, mouse. The possibility that these differences would not be related to the species of origin, but rather to differences between individuals or clonal origin is not likely, since the intraspecific variation in FS was not more than 50% in human fibroblast cultures (Röhme, 1979; Röhme, unpublished data). Interestingly, in the case of these human fibroblasts, the FS appears to be related to the donor age or to the 'age' in culture. Thus, except for

a relation to the phylogenetic differences, the relation to the species of origin found here may also be linked to the life-span of the species of origin of the cells.

It is also noteworthy that except for constitutional differences, the FS potentials vary with the conditions of cultivation (Okada & Murayama, 1966; Röhme, 1979). In fact, different sera or even specific serum components like albumin seem to affect concomitantly both cell fusion and the replicative potential of human fibroblast cells (Todaro & Green, 1964; Ahkong *et al.* 1973; Röhme, 1979).

As to the relation between FS and the species of origin, it is noteworthy that Sendai is a primate virus (Bell, 1965). In an evolutionary context, therefore, it is perhaps not surprising that its potency is higher in human compared to rodent cells. Thus, it is possible that the molecular manifestation(s) of the FS potential may at least partly reside in the cell-surface receptors for Sendai virus, i.e. sialic acid-containing glycoproteins (Wintzler, 1970). The role of the cell surface in relation to cell fusion is also suggested by the preferential fusion of like cells (Mukherjee, Dev & Miller, 1970; Koprowski, 1971), and by the fact that cells in different parts of the cell cycle fuse with different efficiencies (Stadler & Adelberg, 1972). Trypsin removes certain glycoproteins and makes others more accessible (Phillips & Morrison, 1973; Mastro, Beer & Mueller, 1974). Trypsinization also influences the function of the underlying cell membrane/cytoskeleton system (Nicolson, 1973; de Petris, Raff & Mallucci, 1973). The fact that the FS potential was determined on trypsinized cells thus emphasizes that its cellular manifestation primarily resides in the specificity of the cell membrane/cytoskeleton system.

Although the mechanisms that underlie the fusion process are far from being understood, in essence this process is influenced by the membrane 'fluidity', the membrane component mobility and the creation of particle-free areas (Ahkong, Fisher, Tampion & Lucy, 1975; Lucy, 1978; Poste & Pasternak, 1978). According to Ahkong *et al.* (1975), the basic mechanism of fusion would require a perturbation of the bi-layer structure of the membrane lipids that increases the fluidity of the membrane or in extreme cases results in micelle formation (Lucy, 1970). This would lead to fusion of adjacent membranes by allowing the interaction and intermixing of the disturbed lipid molecules of closely apposed membranes. Thus the efficiency of the cell fusion process would be dependent on the types of lipids in the membrane (Ahkong *et al.* 1973; Papahadjopoulos, Poste & Schaeffer, 1973; Papahadjopoulos, Poste, Schaeffer & Vail, 1974; Haywood, 1978) and the readiness by which protein-free lipid areas in the membrane are induced (Lucy, 1978; Nicolson, 1976). The regulatory functions of Ca^{2+} and ATP are also important in this connexion (Okada & Murayama, 1966; Okada, Murayama & Yamada, 1966; Poste & Allison, 1973; Volsky & Loyter, 1978).

Finally, the procedures outlined here offers a simple accurate means to determine genetic or constitutional differences in fusion sensitivity and to evaluate the biological significance of this response. It may also be anticipated that the FS potential in many respects would be beneficial in defining the efficiency of the molecular interactions related to the cell fusion process and may thus be considered as a probe for cell membrane/cytoskeleton function.

I would like to thank Majlis Olin-Fatih and Berit Röhme for excellent technical assistance, and Waheeb Henseen for many valuable discussions, constructive criticism and encouragement throughout this work. I also gratefully acknowledge the excellent technical assistance of the late Elly Lövkvist in the initial phase of this work. My thanks to Per-Olof Mårdh (Institute of Microbiology, University Hospital of Lund) for kindly helping with the mycoplasma tests. This work was supported by the Swedish Cancer Society, the Swedish Natural Science Research Council, the Nilsson-Ehle Foundation and the Augusta Persson Foundation for Medical Research.

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(*Received* 23 *October* 1980)