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THE ROLE OF EXTRACELLULAR CALCIUM IONS IN HVJ (SENDAI VIRUS)-INDUCED CELL FUSION

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Summary

The biochemical and biophysical roles of extracellular calcium ions in HVJ (Sendai virus)-induced cell fusion were studied. (1) Various kinds of cell, such as Ehrlich ascites tumor cells, mouse melanoma cells (B16-CW1 cells) and human epidermoid carcinoma cells (KB cells), could fuse in Ca^{2+}-free medium containing a cheletor, glycoletherdiaminetetraacetic acid, in the same way as in Ca^{2+}-containing medium. (2) The ATP content in Ehrlich ascites tumor cells decreased rapidly when the cells were treated with the virus in Ca^{2+}-free medium but not in Ca^{2+}-containing medium. (3) Intracellular adenine nucleotides leaked out into the reaction medium when the cells were treated with the virus in Ca^{2+}-free medium but not in Ca^{2+}-containing medium. (4) On addition of the virus, O_2 consumption of Ehrlich ascites tumor cells decreased in Ca^{2+}-free medium, but not in Ca^{2+}-containing medium. (5) HVJ (Sendai virus) did not affect production of lactate by Ehrlich ascites tumor cells in both Ca^{2+}-free medium and Ca^{2+}-containing medium. These observations suggest that the role of extracellular Ca^{2+} in virus-induced cell fusion is to maintain the ATP and other intracellular metabolite contents at normal levels instead of triggering the fusion reaction itself.

Introduction

Fusion of biological membrane is an important and fundamental phenomenon in cell life. Many biological processes involve membrane fusion. Ca^{2+} has been shown to be involved in various kinds of membrane fusion phenomena [1]. Ca^{2+} has been also shown to be essential for promoting virus- and chemical-induced cell fusion of mammalian and chicken cells [2—4]. Okada and Murayama [2] previously reported that HVJ (Sendai virus)-induced fusion of

various kinds of line cell was dependent on the presence of Ca^{2+} in the incubation medium and that when the cells were incubated with the virus in the presence of a chelator, EDTA, the cells lysed but did not fuse. Toister and Loyter [3] also previously reported that this virus-induced fusion of chicken erythrocytes was dependent on the presence of Ca^{2+} in the incubation medium. However, very little is known about the role of extracellular Ca^{2+} in the fusion reaction.

We studied the role of Ca^{2+} in the incubation medium in HVJ (Sendai virus)-induced cell fusion. We found that various kinds of cell could fuse on addition of this virus to Ca^{2+}-free medium as well as Ca^{2+}-containing medium and suggested that the role of Ca^{2+} in the incubation medium was to maintain the contents of ATP and other metabolites at normal levels instead of promoting the cell fusion itself.

Materials and Methods

Reagents. Reagents were purchased from the following sources: fetal bovine serum and calf serum from Flow Labs.; *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (Bes) (one of Good's buffers), glycoletherdiaminetetraacetic acid (GEDTA) and EDTA from Dojindo Co.; trizma base and firefly lantern extract from Sigma Co.

HVJ (Sendai virus) was kindly supplied by Professor Y. Okada (Osaka University). The virus was grown in the allantoic sacs of chick embryos. The infected allantoic fluid was harvested and the virus was purified by differential centrifugation in Dulbecco's phosphate-buffered saline without $CaCl_2$ or $MgCl_2$, then suspended in the medium. The HVJ (sendai virus) solution was irradiated with ultraviolet light to reduce the infectious activity without any significant inhibition of the fusion activity [5]. The virus titer was measured in hemagglutinating units (HAU) using Salk's pattern method.

Cells. Strain cells were kindly supplied by the following sources: Ehrlich ascites tumor cells from Dr. T. Morita (Aichi Cancer Center), KB cells (derived from a human epidermoid carcinoma) from Dr. K. Shimada (Aichi Cancer Center) and B16 cells (derived from a mouse melanoma) from Dr. Y. Mishima (Kobe University). The B16-CW1 cells used in this work are a clonal cell line isolated from B16 cells and have no detectable melanin. Ehrlich ascites tumor cells were planted in the abdomen of ddY mice and grown for 7 to 10 days. Stock cultures of KB and B16-CW1 cells were maintained as monolayers in flasks (Falcon, Type 3013) containing 5 ml of culture medium consisting of 90% Eagle's minimum essential medium and 10% fetal bovine serum (for B16-CW1 cells) or calf serum (for KB cells). The cells were incubated at 37°C in a water-saturated atmosphere of 5% CO_2 and 95% air. Subculture was performed every 3 to 7 days with 0.25% trypsin (Difco; 1 : 250) after the cultures had reached confluence.

Reaction media. (1) GEDTA medium A: 115 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 2 mM GEDTA and 10 mM Bes at pH 7.4 (adjusted with NaOH). The final concentration of Na^+ was adjusted to 135 mM by addition of concentrated NaCl.

(2) Ca^{2+} medium A: 115 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 1 mM

$CaCl_2$ and 10 mM Bes at pH 7.4 (adjusted with NaOH). The final concentration of Na^+ was adjusted to 135 mM by addition of concentrated NaCl.

(3) GEDTA medium B: 140 mM NaCl, 54 mM KCl, 0.44 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 2 mM GEDTA and 20 mM Tris-HCl at pH 7.4.

(4) Ca^{2+} medium B: 140 mM NaCl, 54 mM KCl, 0.44 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 1 mM $CaCl_2$ and 20 mM Tris-HCl at pH 7.4.

The concentrations of Ca^{2+} in GEDTA medium A and glucose, kindly measured by Dr. H. Kato (Toa Gosei Co.) with an atomic absorption spectrophotometer, were below 10^{-7} M and below 0.01% (w/w), respectively.

Cell fusion. (1) Monolayer system: KB cells grown as monolayers in Petri dishes (Falcon, 35 mm in diameter) were washed four times with GEDTA medium A or Ca^{2+} medium A, and a volume of less than 40 μl of the HVJ (Sendai virus) solution was added to cultures in 1 ml of GEDTA medium A or Ca^{2+} medium A with gentle shaking to give the final titers of HVJ (sendai virus) indicated in the text. The mixture was incubated at 37°C for 30 to 80 min.

(2) Suspension system: Ehrlich ascites tumor cells were withdrawn from the peritoneal cavity of mice, then washed four to five times with GEDTA medium A or GEDTA medium B to remove contaminating red blood cells, leucocytes, ascites fluids and Ca^{2+}. B16-CW1 cells were removed from the stock flasks with 0.05% EDTA, washed three times with GEDTA medium A, then resuspended in GEDTA medium A or Ca^{2+} medium A. A volume of less than 40 μl of the virus solution was added to 1-ml portions of the suspension of these cells ($1 \cdot 10^7$ cells) to give the final titers of HVJ (Sendai virus) indicated in the text. After the resulting suspension had been kept on an ice/water bath for 10 min to complete the virus-induced aggregation of the cells, it was incubated at 37°C for 30 min in a shaking bath of 180 vibrations/min. The suspension was then cooled in an ice/water bath. The number of cells was counted before and after the fusion reaction by the method of Okada and Tadokoro [5]; the degree of cell fusion was proportional to the degree of decrease in cell number. Thus, the total number of cells in the test sample was directly compared with that of the control containing no virus to estimate the degree of cell fusion. Lysis of the cells was checked with erythrosin B by the method of Phillips and Terryberry [6].

Measurement of ATP content. 1 ml of the suspension of Ehrlich ascites tumor cells was extracted with $HClO_4$ (final concentration 6%) and neutralized with K_2CO_3. The ATP content of the neutralized extract was measured by the firefly luciferase method [7] with a liquid scintillation spectrometer (Aloka, LSC) using a ^3H channel.

Measurement of leakage of substances having A_{260}. After the suspension of Ehrlich ascites tumor cells had been treated with the virus, it was centrifuged and the resulting supernatant was mixed with a portion of 12% perchloric acid. After centrifugation, the absorbance at 260 nm of the supernatant was measured with a spectrophotometer. The chemical components of the supernatant were analyzed by Dowex-1 column chromatography.

Measurement of O_2 consumption. O_2 consumption of the cells was measured manometrically. 2 ml cell suspension were preincubated for 10 min at 37°C, then mixed with the virus (final concentration 2000 HAU/ml) in the side arm.

Measurement of lactate content. A suspension of Ehrlich ascites tumor cells

was extracted and neutralized as described in the case of ATP. The lactate content of the neutralized extract was measured by the method of Barker and Summerson [8].

Measurement of protein amount. Protein amount was measured by the method of Lowry et al. [9].

Electron microscopy. The cells incubated with HVJ (Sendai virus) were centrifuged, fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) and postfixed with 2% buffered osmium tetraoxide. After dehydration, the cells were embedded in Epon and stained with uranyl acetate and lead citrate. Thin sections were cut on a JEOL JUM-7 and viewed in a JEOL 100CX electron microscope.

Results

Nonrequirement of extracellular Ca^{2+} for cell fusion

KB cells grown as monolayers started to fuse in Ca^{2+} medium A within 10 min after addition of 2000 or 4000 HAU/ml of virus, and multinucleated giant sheets of cells were formed within 60 min after the addition (Fig. 1b). In GEDTA medium A, KB cells grown as monolayers became spherical and detached from the surface of Petri dishes regardless of the presence or absence

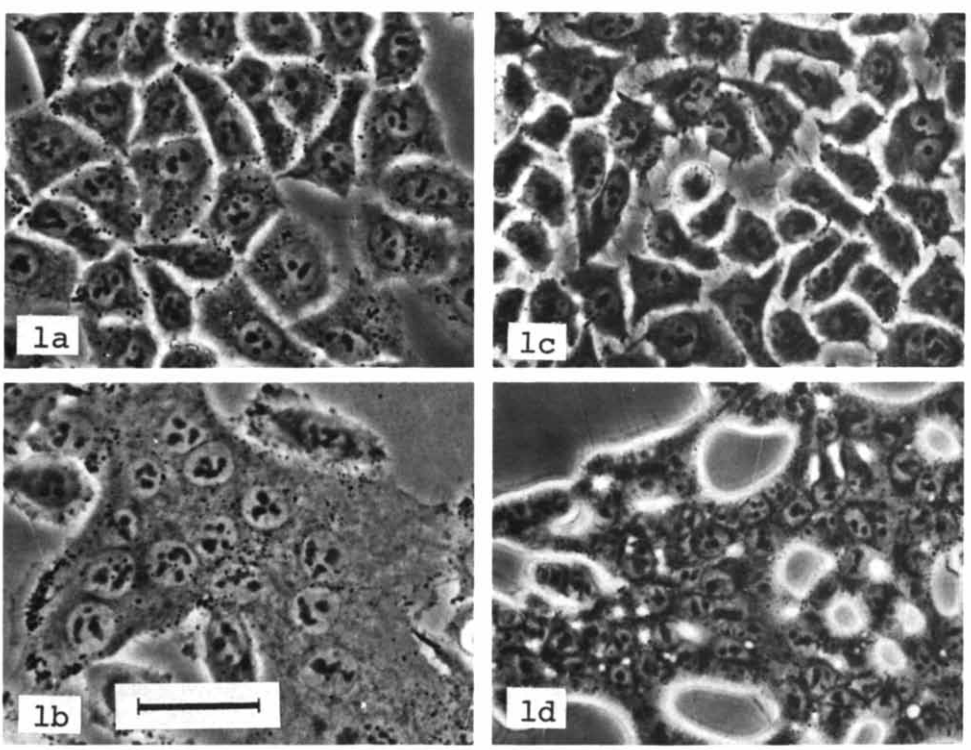


Fig. 1. Phase-contrast micrographs of HVJ (Sendai virus)-induced fusion of human epidermoid carcinoma cells (KB cells) in Ca^{2+} medium A (a,b) and GEDTA medium A (c,d). (a,c), 5 min after incubation of the cells in the absence of the virus; (b,d), 60 min after addition of 4000 HAU/ml of the virus. Bar, 40 μm.

of the virus. However, these cells also fused on addition of 2000 or 4000 HAU/ml of the virus (Fig. 1d). No significant cell lysis was observed under phase-contrast microscopy. When the fused cells formed in GEDTA medium A were incubated in culture medium (Eagle's minimum essential medium supplemented with calf serum), they became reattached and spread on the surface of the Petri dishes again.

Ehrlich ascites tumor cells also fused in GEDTA medium A as well as in Ca^{2+}

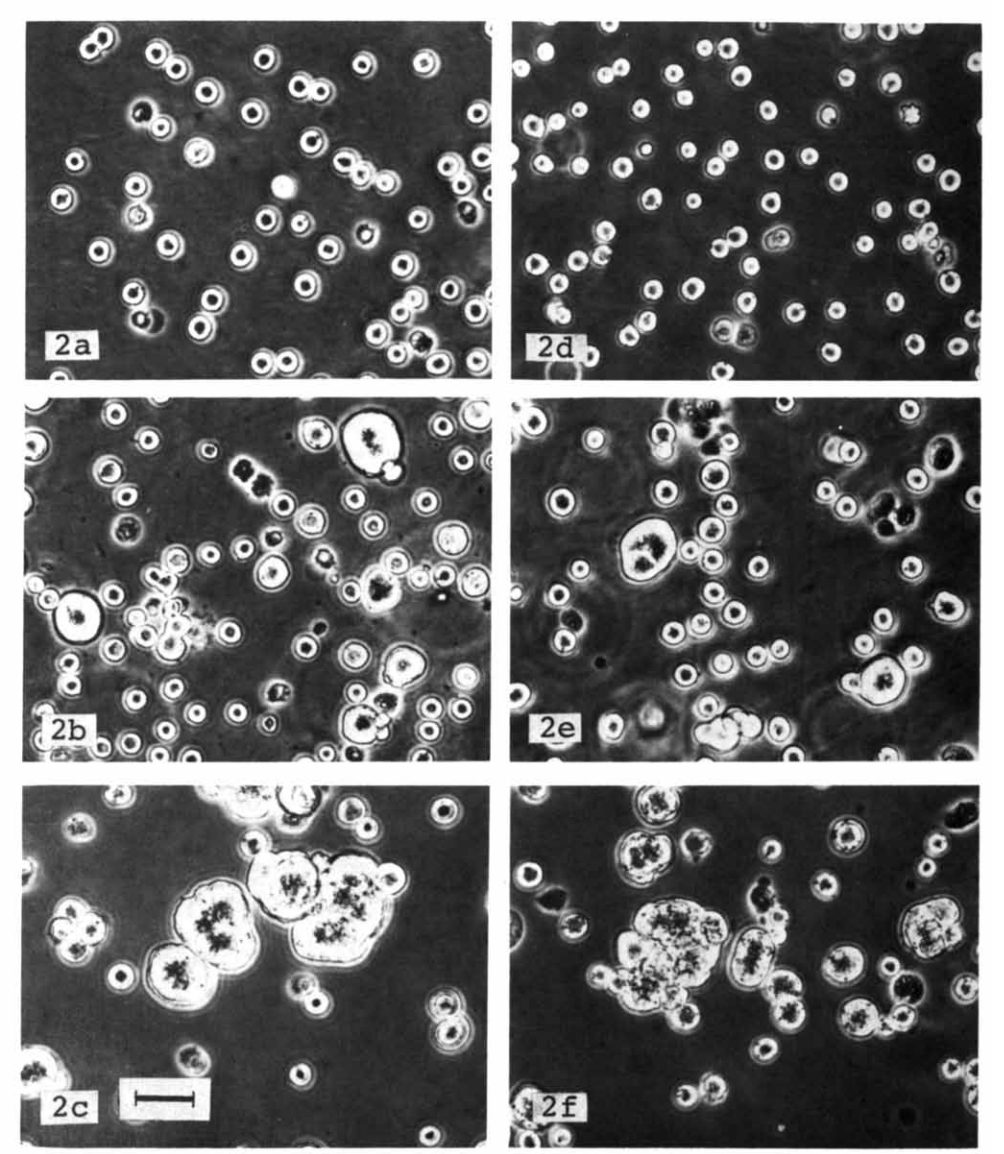


Fig. 2. Phase-contrast micrographs of HVJ (Sendai virus)-induced fusion of Ehrlich ascites tumor cells in Ca^{2+} medium A (a,b,c) and GEDTA medium A (d,e,f). (a,d), 30 min after incubation of the cells in the absence of the virus; (b,e) and (c,f), 30 min after addition of 2000 and 4000 HAU/ml of the virus, respectively. Bar, 40 μm.

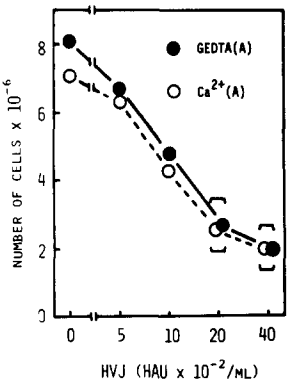


Fig. 3. Nonrequirement of extracellular Ca^{2+} in HVJ (Sendai virus)-induced fusion of Ehrlich ascites tumor cells. Fusion frequency (number of cells) was counted 30 min after addition of 500 to 4000 HAU/ml of the virus to Ca^{2+} medium A (Ca^{2+} (A)) (○) or GEDTA medium A (GEDTA (A)) (●). The data are from one typical experiment. The same results were obtained in three independent experiments.

medium A on addition of more than 500 HAU/ml of HVJ (Sendai virus) (Figs. 2 and 3). There was no significant difference in the fusion frequency between GEDTA medium A and Ca^{2+} medium A in the range of virus titer from 500 to 4000 HAU/ml. On addition of less than 2000 HAU/ml of the virus, lysis of only a few cells was observed under phase-contrast microscopy in both GEDTA medium A and Ca^{2+} medium A (Fig. 2). On addition of 4000 HAU/ml of the virus, about 30% of the Ehrlich ascites tumor cells was lysed in GEDTA medium A but not in Ca^{2+} medium A. Moreover, B16-CW1 mouse melanoma cells fused in GEDTA medium as well as in Ca^{2+} medium on addition of 4000 HAU/ml of the virus and no cell lysis was observed in both GEDTA medium A and Ca^{2+} medium A.

Effect of glucose on cell fusion

The percentages of the lysed cells in GEDTA medium A on fusion were much less than those previously reported by Okada and Murayama [2]. They found that Ehrlich ascites tumor cells did not fuse but more than 90% became lysed on addition of more than 1000 HAU/ml of HVJ (Sendai virus) in Ca^{2+}-free 'balanced salts solution' buffer 1, which contained 140 mM NaCl, 54 mM KCl, 0.34 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 10 mM Tris-HCl and 1 mM EDTA. Unlike our GEDTA medium A, it contained no glucose, was buffered with Tris-HCl and phosphate, chelated with EDTA, and was high in KCl concentration. We confirmed that Ehrlich ascites tumor cells did not fuse but lysed by treatment with virus in Ca^{2+}-free buffer 1 as reported by Okada and Murayama [2] (Fig. 4c). We examined what chemical(s) in the Ca^{2+}-free buffer 1 affects cell lysis during treatment with the virus, and found that when Ehrlich ascites tumor cells were treated with 4000 HAU/ml of the virus in Ca^{2+}-free buffer 1 supplemented with 5.5 mM glucose or GEDTA medium B supplemented with 5.5 mM glucose, the cells fused without significant cell lysis (Fig. 4d) and that when the cells were treated with 4000 HAU/ml of the virus in GEDTA medium

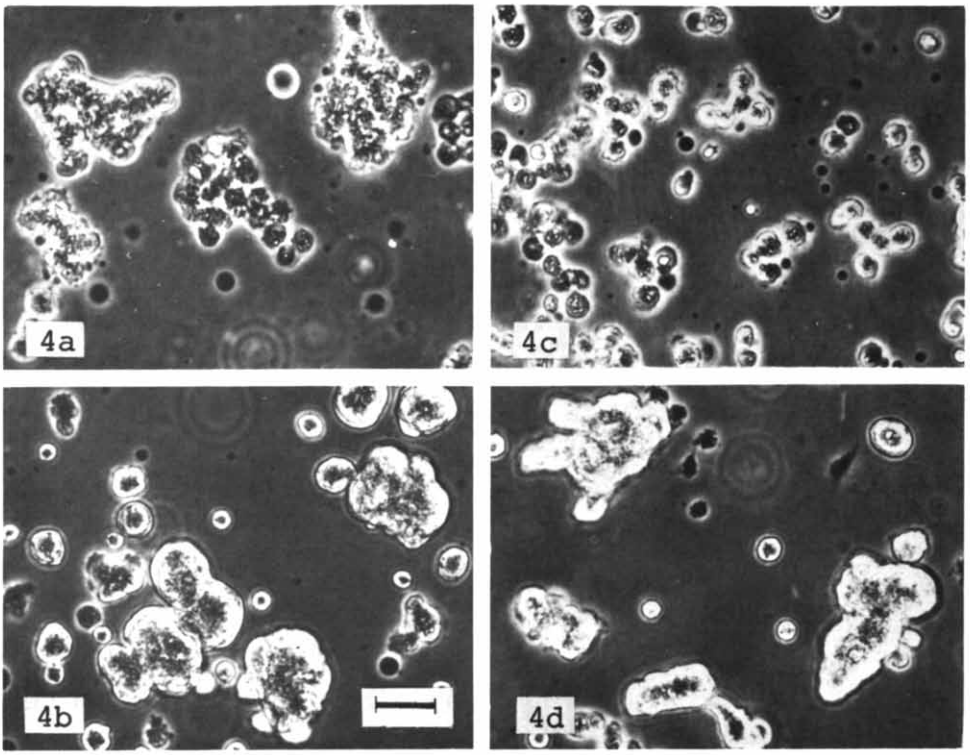


Fig. 4. Phase-contrast micrographs of the effect of glucose in GEDTA medium on HVJ (Sendai virus)-induced fusion of Ehrlich ascites tumor cells. a, b, c and d: 30 min after addition of 4000 HAU/ml of the virus to glucose-free GEDTA medium A, glucose-containing GEDTA medium A, glucose-free GEDTA medium B and glucose-containing GEDTA medium B, respectively. Bar, 40 μm.

A containing no glucose, the cells did not fuse but lysed (Fig. 4a). These observations show that glucose is necessary for cell fusion in GEDTA medium. The fusion frequencies of Ehrlich ascites tumor cells in glucose-containing GEDTA medium B and glucose-containing Ca^{2+} medium B were the same as those in GEDTA medium A and Ca^{2+} medium A, respectively, thus, we used medium B in the following experiments.

TABLE I

EFFECTS OF SOME REAGENTS ON FUSION OF EHRLICH ASCITES TUMOR CELLS IN GEDTA MEDIUM B

HVJ (Sendai virus) 4000 HAU/ml; (+), Fusion; (—), No fusion; n.d.: Not determined. Percentages of lysis are from one typical experiment. The same results were obtained in three independent experiments.

Reagents (5.5 mM)	Fusion	Lysis
No addition	—	94%
Glucose	+	23%
Pyruvate	+	56%
3-*O*-Methyl glucoside	—	n.d.
2-Deoxyglucose	—	n.d.
Sucrose	—	94%
Sorbitol	—	85%

Glucose analogs, such as 3-*O*-methyl glucoside and 2-deoxyglucose, sucrose and sorbitol (5.5 mM, respectively), could not substitute for glucose in cell fusion in GEDTA medium B. Addition of pyruvate in GEDTA medium B partially prevented cell lysis and stimulated cell fusion (Table I).

Decrease of ATP content of Ehrlich ascites tumor cells in Ca^{2+}-free media during the fusion reaction

The ATP content of Ehrlich ascites tumor cells did not change significantly during cell fusion in Ca^{2+}-medium B regardless of the presence or absence of glucose. However, the ATP content decreased rapidly in GEDTA medium B on addition of HVJ (Sendai virus): the ATP content in glucose-free GEDTA medium B became one-twentieth of the normal content and the ATP content in glucose-containing GEDTA medium B became one-fourth of the normal content after 20 min incubation at 37°C. This observation shows that supplementation of glucose to the GEDTA medium partially prevented the decrease of ATP content (Fig. 5).

Leakage of adenine nucleotides from Ehrlich ascites tumor cells

Fig. 6 shows the leakage of substances which are acid-soluble and have optical absorbance at 260 nm during the fusion reaction. When the cells were treated with HVJ (Sendai virus) in glucose-containing Ca^{2+} medium B or glu-

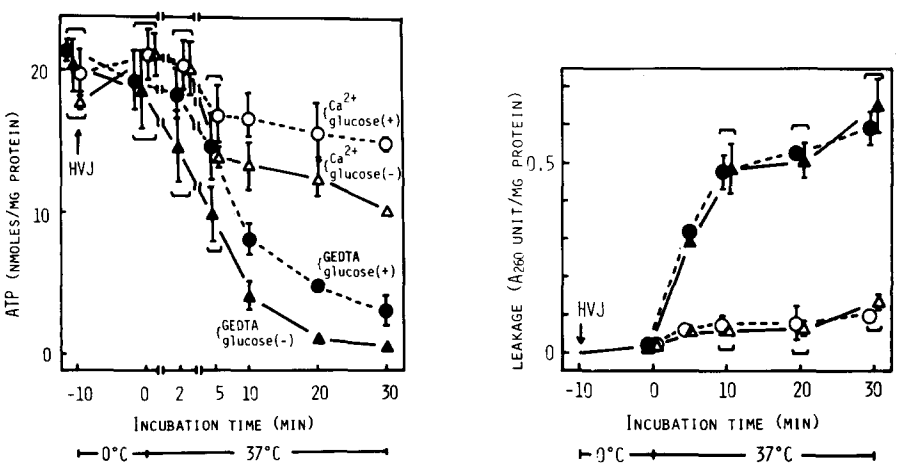


Fig. 5. Changes of ATP contents in Ehrlich ascites tumor cells during fusion reaction. 4000 HAU/ml of the virus was added to glucose-containing GEDTA medium B (●), glucose-free GEDTA medium B (▲), glucose-containing Ca^{2+} medium B (○) and glucose-free Ca^{2+} medium B (△) at −10 min. After the mixture had been allowed to stand for 10 min in an ice/water bath, the mixture was incubated at 37°C in a water bath with shaking. The data are averages of three experiments. The vertical bars represent S.E.

Fig. 6. Leakage of acid-soluble substances having absorbance at 260 nm from Ehrlich ascites tumor cells during fusion reaction. The ordinate indicates the absorbance units at 260 nm. One unit of absorbance at 260 nm is the amount of material per ml of solution which gives one absorbance at 260 nm in a cell of 1-cm light path. The virus (2000 HAU/ml) was added to glucose-containing GEDTA medium B (●), glucose-free GEDTA medium B (▲), glucose-containing Ca^{2+} medium B (○) and glucose-free Ca^{2+} medium B (△) at −10 min. After the mixture had been allowed to stand for 10 min in an ice/water bath, the mixture was incubated at 37°C in a water bath with shaking. The data are averages of four experiments. The vertical bars represent S.E.

cose-free Ca^{2+} medium B, no significant leakage was seen. However, when the cells were treated with the virus in glucose-containing GEDTA medium B or glucose-free GEDTA medium B, the substances leaked out from the cells. Glucose did not inhibit the leakage. In the absence of the virus, no leakage occurred in GEDTA medium B as well as Ca^{2+} medium B. Chemical analysis of the leaked substances by Dowex-1 column chromatography showed that 31% of the absorbance was due to 5'-AMP and 14% to ADP. Thus, one unit of the absorbance at 260 nm meant 22 nmol of ADP and 9 nmol of 5'-AMP.

Decrease of O_2 consumption of Ehrlich ascites tumor cells during fusion reaction in Ca^{2+}-free media

O_2 consumption of Ehrlich ascites tumor cells in Ca^{2+} medium B did not decrease with addition of 2000 HAU/ml of HVJ (Sendai virus) (Fig. 7a). Addition of glucose did not affect the O_2 consumption.

O_2 consumption of the cells in GEDTA medium containing no virus was slightly low compared with that in Ca^{2+} medium B containing no virus. O_2 consumption of the cells in GEDTA medium B decreased with addition of 2000 HAU/ml of the virus (Fig. 7b). In glucose-free GEDTA medium B, the decrease was remarkable and the cells consumed hardly any O_2 from 10 min after addition of the virus and lysed without fusion. In glucose-containing GEDTA medium B, the decrease was less than that in glucose-free GEDTA medium B and thus the rate from 10 to 20 min was approximately half of that of the virus-free control.

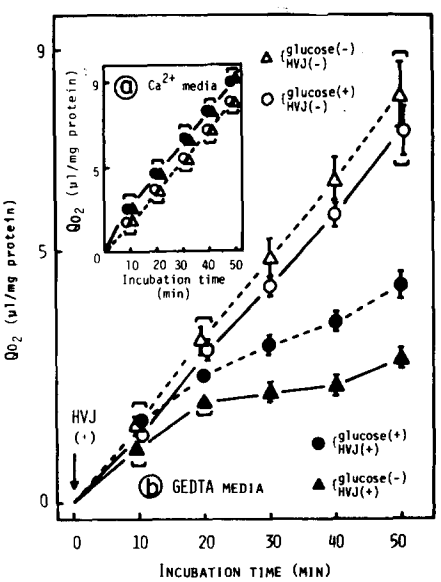


Fig. 7. O_2 consumption of Ehrlich ascites tumor cells during fusion reaction. a shows O_2 consumption of the cells in Ca^{2+} medium B. The virus (2000 HAU/ml) was added to glucose-containing Ca^{2+} medium B (●) and glucose-free Ca^{2+} medium B (▲). No virus was added to glucose-containing Ca^{2+} medium B (○) and glucose-free Ca^{2+} medium B (△). b shows the O_2 consumption of the cells in GEDTA medium B. The virus (2000 HAU/ml) was added to glucose-containing GEDTA medium B (●) and glucose-free GEDTA medium B (▲). No virus was added to glucose-containing GEDTA medium B (○) and glucose-free GEDTA medium B (△). The data are averages of four experiments. The vertical bars represent S.E.

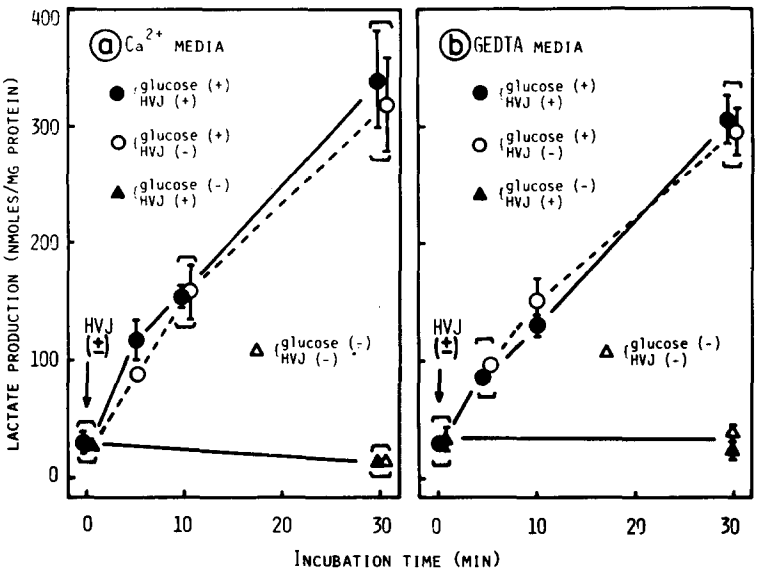


Fig. 8. Lactate production by Ehrlich ascites tumor cells during fusion reaction. a shows lactate production by the cells in Ca^{2+} medium B. The virus (2000 HAU/ml) was added to glucose-containing Ca^{2+} medium B (●) and glucose-free Ca^{2+} medium B (▲). No virus was added to glucose-containing Ca^{2+} medium B (○) and glucose-free Ca^{2+} medium B (△). b shows lactate production by the cells in GEDTA medium B. The virus (2000 HAU/ml) was added to glucose-containing GEDTA medium B (●) and glucose-free GEDTA medium B (▲). No virus was added to glucose-containing GEDTA medium B (○) and glucose-free GEDTA medium B (△). The data are averages of three experiments. The vertical bars represent S.E.

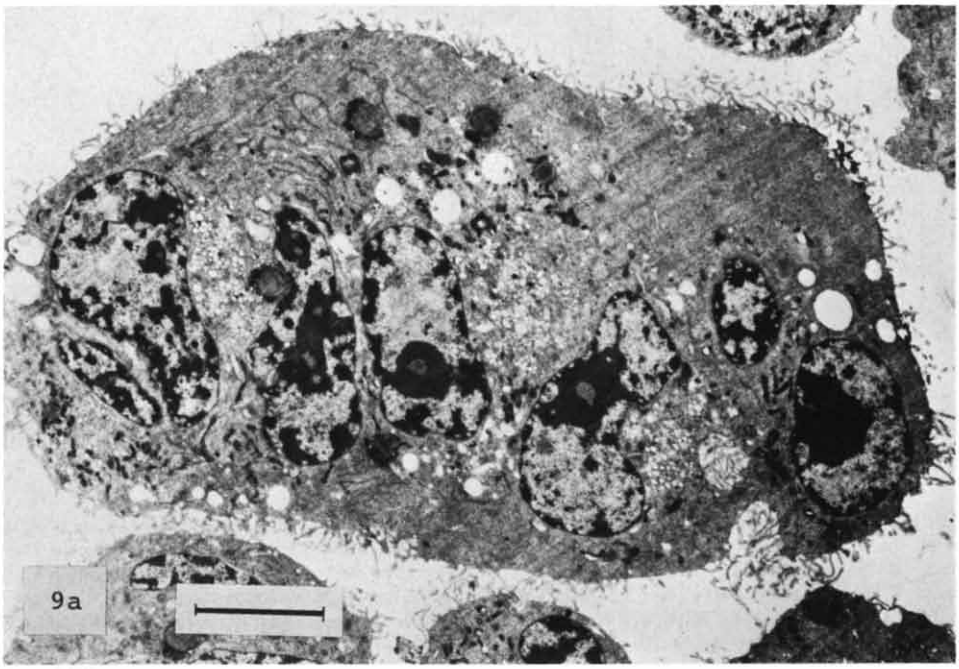
Production of lactate by Ehrlich ascites tumor cells during the fusion reaction

The production of lactate by Ehrlich ascites tumor cells was measured in various media (Fig. 8a,b). The production was dependent on the presence of glucose but independent of the presence of the virus up to 30 min at 37°C incubation. In the presence of glucose, lactate was produced in proportion to the incubation time for 30 min at 37°C in both glucose-containing Ca^{2+} medium B and glucose-containing GEDTA medium B.

Electron microscopic observation of the fused Ehrlich ascites tumor cells in Ca^{2+}-free medium

Fig. 9 shows the electron micrograph of the fused Ehrlich ascites tumor cells formed in the glucose-containing GEDTA medium B with addition of HVJ (Sendai virus). The fused cells were multinucleated and ellipsoidal in shape (approx. 35 μm across the major axis), whereas nonfused cells usually had mononucleus and were spherical in shape and about 13 μm in diameter. The morphology of the fused cells in glucose-containing GEDTA medium B resembled that of the fused cells in glucose-containing Ca^{2+} medium B.

Kim and Okada [10,11] have studied the change of morphology in mitochondria during the fusion reaction using a medium containing Ca^{2+}. They found that the morphology of the mitochondrial matrix transiently changed from an orthodox to a dense contracted conformation on addition of the virus,



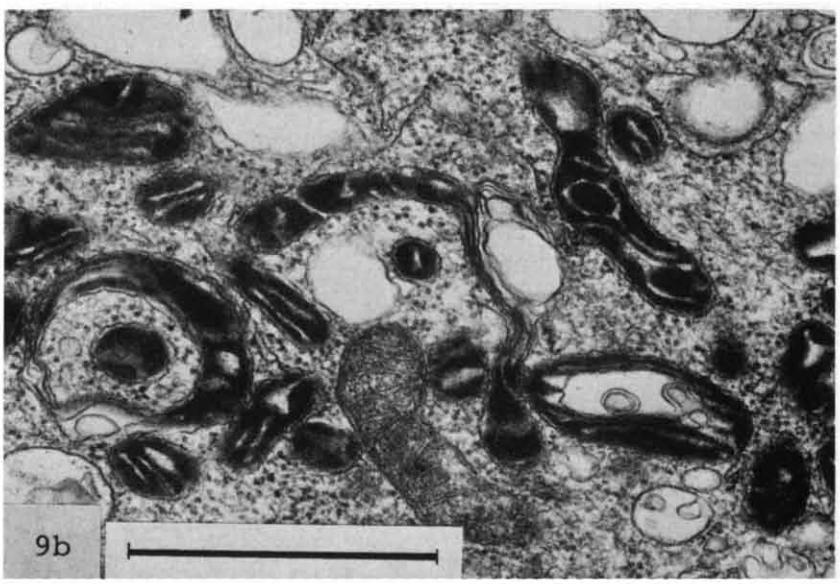


Fig. 9. Electron micrographs of fused Ehrlich ascites tumor cells formed by treatment with HVJ (Sendai virus) in glucose-containing GEDTA medium B. The cells were fixed 20 min after addition of 2000 HAU/ml of the virus. Fig. 9a shows a fused multinucleated cell. Bar, 5 μm. Fig. 9b shows mitochondria in a fused cell. Almost all mitochondria were slender with a dense contracted matrix. A few of them had an orthodox conformation. Scale: 1 μm.

and that the orthodox conformation was restored by further incubation. The same change of mitochondrial morphology was also observed in glucose-containing GEDTA medium B. But unlike in glucose-containing Ca^{2+} medium, orthodox conformation was not restored on further incubation, at least up to 20 min in the GEDTA medium B (Fig. 9b).

Discussion

Nonrequirement of extracellular Ca^{2+} in the fusion reaction

Ca^{2+} in the incubation medium has been described as being essential for HVJ (Sendai virus)-induced fusion of various kinds of mammalian and chicken cells [2,3], inspite of some exceptions [12,13]. Much attention has been focused on the role of extracellular Ca^{2+} as well as that of intracellular Ca^{2+} in this virus-induced cell fusion [14—16]. Yanovsky and Loyter [12] reported in 1972 that extracellular Ca^{2+} was not required for this virus-induced fusion of Ehrlich ascites tumor cells in the presence of ouabain or in a high KCl medium. But Volsky and Loyter [14] reported recently that this virus-induced fusion between the plasma membranes of chicken erythrocytes was dependent on the influx of extracellular Ca^{2+} and discussed that entry of Ca^{2+} into the cells might cause depolymerization of microtubules, which might be a prerequisite for induction of fusion of chicken erythrocytes or of other nucleated cells.

Ohki et al. [15] reported that stimulation of fusion of Ehrlich ascites tumor cells by theophylline was dependent on the extracellular concentration of Ca^{2+}. They also found that ruthenium red abolished this stimulation and the inhibition of the cell fusion by cytochalasin D was influenced by the extracellular concentration of Ca^{2+}. Based on these observations, they discussed the role of mutual interaction of microfilaments, cyclic AMP and Ca^{2+} influx in HVJ (Sendai virus)-induced cell fusion. Recently, Poste and Pasternak [16] proposed extracellular Ca^{2+}-induced lateral phase separation of membrane phospholipids as a possible mechanism for virus-induced cell fusion.

In the present work, we showed that various kinds of mammalian cell, such as Ehrlich ascites tumor cells, B16 mouse melanoma cells and KB cells, fused when the virus was added to a medium containing GEDTA in the same way as in a medium containing Ca^{2+}. This result shows clearly that extracellular Ca^{2+} is not requisite for virus-induced cell fusion and thus neither influx of extracellular Ca^{2+} nor Ca^{2+}-induced lateral phase separation of membrane phospholipids is needed for virus-induced fusion of various mammalian cells. However, these results do not exclude the possibility that the intracellular Ca^{2+} may play an important role in this virus-induced cell fusion.

Role of extracellular Ca^{2+} in HVJ (Sendai virus)-induced cell fusion

When Ehrlich ascites tumor cells were incubated with HVJ (Sendai virus) for 20 min in the absence of extracellular Ca^{2+}, about 11 nmol/mg protein of ADP and 5 nmol/mg protein of 5'-AMP (calculated from the measurement of A_{260}, chemical analysis and molecular extinction coefficients) leaked out (see Fig. 6). The total content of adenine nucleotides in Ehrlich ascites tumor cells has been estimated to be approx. 25 nmol/mg protein [17]. Thus, the percentage of leaked adenine nucleotides was calculated to be approx. 64%. On the other

hand, when Ehrlich ascites tumor cells were incubated with HVJ (Sendai virus) in the presence of extracellular Ca^{2+}, only 2.6 nmol/mg protein of adenine nucleotides leaked out.

The intracellular ATP content of Ehrlich ascites tumor cells decreased during treatment with the virus in the absence of extracellular Ca^{2+}, but not in the presence of extracellular Ca^{2+} (see Fig. 5). One possible reason for the decrease in ATP content is the leakage of adenine nucleotides (see Fig. 6). Another possible reason is the decrease in the activity of oxidative phosphorylation during the fusion reaction in Ca^{2+}-free medium (see Fig. 7).

The cells in a medium containing no glucose and Ca^{2+} did not fuse but lysed on addition of HVJ (Sendai virus). The cell lysis may be due to the decrease in intracellular ATP content caused by leakage of adenine nucleotides and suppression of the activity of oxidative phosphorylation. The suggestion that intracellular ATP is important for virus-induced cell fusion was previously reported by Okada et al. [18] and Yanovsky and Loyter [12]. The present observations on the role of ATP on this virus-induced cell fusion support their original finding.

The cells in a medium containing Ca^{2+} fused without cell lysis on addition of HVJ (Sendai virus) regardless of the presence or absence of glucose. The role of Ca^{2+} seems to be to maintain the intracellular ATP content during the fusion reaction by suppressing leakage of adenine nucleotides and maintaining the activity of oxidative phosphorylation.

Role of glucose in HVJ (Sendai virus)-induced cell fusion

The cells, such as Ehrlich ascites tumor cells, could fuse without cell lysis on addition of HVJ (Sendai virus) to Ca^{2+}-free medium when the medium was supplemented with glucose. Glucose analogs, such as 3-*O*-methyl-glucoside and 2-deoxyglucose, sucrose and sorbitol could not substitute for glucose in the fusion reaction in Ca^{2+}-free medium. Pyruvate could partially substitute for glucose (see Table I). The glycolytic activity of Ehrlich ascites tumor cells was maintained during the fusion reaction regardless of the presence or absence of extracellular Ca^{2+} (see Fig. 8). These observations show that glucose is used as an energy source for ATP synthesis. Glucose did not suppress the leakage of adenine nucleotides during the fusion reaction in Ca^{2+}-free medium (see Fig. 6). This observation shows that even under conditions where adenine nucleotides leak out from the cells, the cells can fuse without cell lysis when the glycolytic activity is maintained.

The cells in a meidum containing no glucose and Ca^{2+} did not fuse but lysed on addition of the virus. The cell lysis may be due to the decrease in intracellular ATP content caused by leakage of adenine nucleotides and suppression of the activities of both oxidative phosphorylation and glycolysis.

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