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SHORT COMMUNICATION

EFFECT OF CALCIUM ON THE CELL FUSION REACTION CAUSED BY HVJ

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As reported previously, when Hemagglutinating Virus of Japan (HVJ) is added to Ehrlich ascites tumor cells (ETC), the virus particles are promptly adsorbed onto the ETC and these cells agglutinate together at 4°C. The agglutinating cells then fuse together within 30 minutes during incubation at 37°C under aerobic conditions. In routine work, we have used a modified Hanks' solution containing calcium and magnesium ions as the medium for the cell fusion reaction (OKADA and TADOKORO, 1962). Recently, it was observed that when Ca ions were removed from this medium the cell fusion reaction did not occur.

The Z strain of HVJ used was propagated in embryonated eggs and HVJ was prepared from the infected chorioallantoic fluid by differential centrifugation. It was suspended in BSS (NaCl 1.4×10^{-1} M, KCl 5.4×10^{-2} M, Na_2HPO_4 3.4×10^{-4} M, KH_2PO_4 4.4×10^{-4} M, buffered with 10^{-2} M of Tris-HCl to pH 7.6). Ca ions contaminating the virus sample were removed by passage of the sample through a column of Sephadex G-200.

One half ml of a 10 per cent suspension of ETC in BSS, 0.5 ml of 2,500 HAU/ml of HVJ suspended in BSS and 0.5 ml of various concentrations of $CaCl_2$ dissolved in BSS were mixed in test tubes and kept at 4°C for 10 minutes. During this period, ETC agglutinated together and many cell clumps appeared in every tube containing HVJ. These tubes

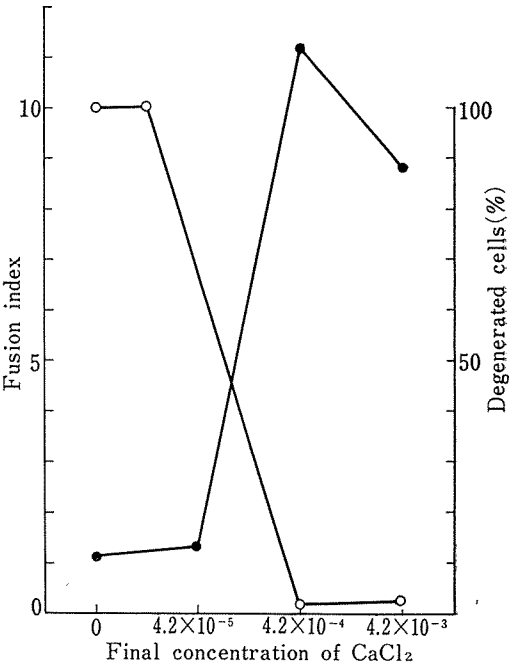

FIGURE 1 Effect of calcium on the cell fusion reaction. One half ml of a 10% suspension of ETC in BSS, 0.5 ml of 2,500 HAU/ml of HVJ suspended in BSS and 0.5 ml of variable concentrations of $CaCl_2$ in BSS were mixed in test tubes and incubated at 37°C for 60 minutes with shaking. The F.I. (fusion index) was calculated by the following formula (OKADA and TADOKORO, 1962),

$$\text{F.I.} = \frac{\text{cell number in control without virus}}{\text{cell number in test tube}} - 1.0$$

●——● F.I.

○——○ Per cent of degenerated cells

were incubated at 37°C for 60 minutes with shaking and then cooled and observed microscopically.

As indicated in Fig. 1, the cell fusion reaction scarcely occurred and the cells were almost completely degenerated in the tubes containing no Ca and only 4.2×10^{-5}M of $CaCl_2$. However, the control (virus-free and Ca-free) appeared intact after the incubation at 37°C. In the tubes containing 4.2×10^{-4}M and 4.2×10^{-3}M of $CaCl_2$, typical cell fusion was observed and no cell degeneration was found.

When 1 mM of EDTA (final concentration) was added to the sample containing 4.2×10^{-4}M $CaCl_2$ before incubation at 37°C, the cell fusion reaction was inhibited and all cells degenerated during incubation at 37°C for 60 minutes just as in the Ca-free medium. This inhibition by EDTA was reversed by addition of sufficient $CaCl_2$. Mg ions could not replace Ca ions and they inhibited the cell fusion reaction.

The cell fusion reaction was carried out at 37°C in a series of tubes of a BSS medium containing 4.2×10^{-2}M of $CaCl_2$ and 1 mM of EDTA was added at appropriate intervals. Then the samples were each reincubated at 37°C for 60 minutes. These samples were then cooled and one part of each sample was used for microscopical observation while the other part was centrifuged at 2,000 rpm for 20 minutes and the protein content of the supernatant was determined by Lowry's method. The results are shown in Fig. 2. In the sample in which EDTA was added before incubation at 37°C, there was scarcely any cell fusion, all the cells degenerated considerably and protein was liberated from the cells into the medium. As the period before addition of EDTA during the cell fusion reaction at 37°C was increased, the degree of cell fusion increased (this is indicated by the decrease in the total cell number shown in Fig. 2), while the degree of cell degeneration decreased and the liberation of protein was inhibited with increase in the period before addition of EDTA. Intact cells were first seen in the sample to which EDTA was added after 5 minutes and the cell number gradually increased with increase in the period before EDTA addition reaching a plateau when this period was 10-12.5 minutes. These intact cells were almost all fused giant cells, though a few were mononuclear cells. The giant cells in the tubes to

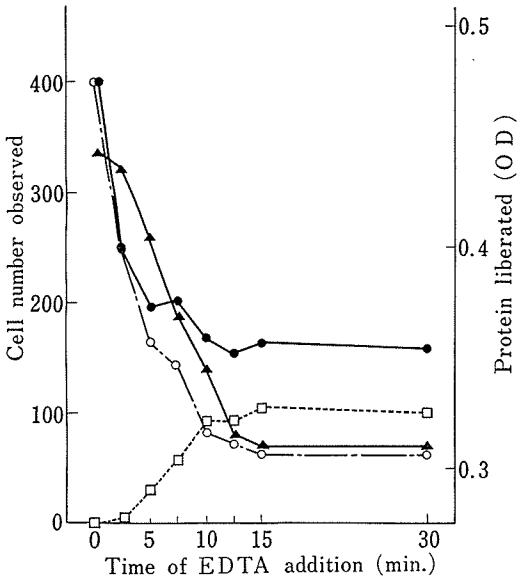

FIGURE 2 Effect of EDTA on the cell fusion reaction. One half ml of a 10% suspension of ETC and 0.5 ml of 2,500 HAU/ml of HVJ, both suspended in BSS containing 4.2×10^{-4}M of $CaCl_2$ were mixed in a series of test tubes and incubated at 37°C with shaking to allow the cell fusion reaction to proceed. One mM of EDTA (final concentration) was added at intervals of 2.5 minutes during the course of the cell fusion reaction to the series of tubes which were then each reincubated at 37°C for a further 60 minutes. After the incubation, one part of each sample was used for microscopical observation and the numbers of degenerated cells, and intact cells were determined. The other part was centrifuged at 2,000 rpm for 20 minutes and the protein content of the supernatant was estimated by Lowry's method as the OD_{700} value.

●——● Total number of cells
○– – –○ Number of degenerated cells
□– – – –□ Number of intact cells
▲——▲ Protein in the medium

which EDTA was added early were not spherical, while the giant cells in the sample to which EDTA was added after 30 minutes were typically spherical. As controls, ETC alone or giant cells appearing after completion of the cell fusion reaction were tested but they were not affected morphorogically by EDTA and there was no liberation of protein from their cytoplasm into the medium on incubation at 37°C for 60 minutes.

These results would indicate the presence of the following 4 steps in the cell fusion reaction, I) adsorption of virus particles onto cells and agglutination of the cells at 4°C, II) disconnection of the cell surface by the action of the virus at 37°C, III) reconnection of the disconnected sites, and IV) spherical giant cell formation. In step III, the fusion of the cell surface of one cell with that of another cell and the reconnection of the cell's own disconnected sites are also completed.

On the basis of the above, steps I and II progress irrespective of whether Ca ions are present or not, while Ca ions are essential for step III, because cell fusion did not occur and the cells degenerated in Ca-free medium. The cell fusion reaction requires energy which is probably supplied from ATP and this energy may be mainly utilized at step III (OKADA, 1962; OKADA *et al.*, unpublished data). From these considerations it is assumed that the activity of cell fusion of HVJ is associated with steps I and II, and when a part of the cell surface structure is disconnected by HVJ, ATPase is activated and reconnection of the disconnected sites involves the utilization of ATP. Ca ions may play a role as activators of ATPase or may be an essential factor in preservation of the demonstrable structure for reconnection.

References

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