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Effects of ethanol on spore germination and cell growth of *Dictyostelium discoideum*

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Effects of ethanol at relatively low concentrations on the spore germination and cell growth of *Dictyostelium discoideum* were examined.

Ethanol at the concentrations lower than 1% did not give any effect on the germination of spores of the strain NC4. When ethanol was given to spores at 2%, the spore germination was prevented by 69% of the control, and the treatment with 5% ethanol completely inhibited the germination.

The effect of ethanol on the cell growth was somewhat different between two strains of NC4 and A3. The dose-dependent effect of ethanol on the cell growth was observed with the cells of the strain NC4. On the contrary, the A3 cells could grow at almost normal rate in the presence of ethanol at concentrations lower than 1% after a certain lag-period. In the medium containing 2% ethanol, the cells of both the strains could proliferate once, but the succeeding cell growth was completely inhibited.

The possible mechanisms for the action of ethanol of *Dictyostelium* cells are discussed.

My recent study shows that a low concentration of ethanol causes almost complete inhibition of the morphogenetic development of the cellular slime mold *Dictyostelium discoideum*, but does not affect the aggregation of cells (HASE, manuscript in preparation). I have also obtained evidence that ethanol perturbs the normal changes in phospholipid composition during development of this organsim. The inhibition of morphogenesis by ethanol seems to be due to the perturbation of the phospholipid composition in cells.

To obtain further information about the effects of ethanol on the development of *D. discoideum*, I investigated the spore germination and cell growth in the presence of ethanol. The germination of spores and the proliferation of cells in the strain NC 4 were slightly inhibited in the presence of 1% ethanol. The growth rate of the A 3 cells was not decreased with ethanol of less than 1%, although the lag-period of growth was prolonged. Two per cent ethanol exerted an inhibitory effect on the cell growth in both the strains.

Materials and Methods

Organisms

D. discoideum strains NC4 and A3 (axenic type) were used in the experiments to test the cell growth. In the experiments on the spore germination, the strain NC4 was used.

Synchrony of spore germination

The germination of spores was synchronized using the technique described by COTTER and RAPER (1968) with a minor modification by YAGURA and IWABUCHI (1976). Amoeba emergence began to occur about 2 h after heat-shocking. Almost all the spores produced myxamoebae 4.5～5 h after the onset of incubation of spores.

In the ethanol treatment of germinating spores, the heat-shocked spores were collected, resuspended at the density of $2\sim3\times10^7$ spores/m*l* of culture in 10 mM potassium phosphate buffer (pH 6.5) containing various concentrations of ethanol and 250 μg/m*l* streptomycin sulfate, and allowed to germinate at 23°C in a shaker. The number of germinated spores was counted with a hemacytometer under a microscope 6 h after the onset of incubation at 23°C.

Culture and ethanol treatment of organism

Amoeba cells of the strain NC4 were grown at 23°C in a liquid medium of 14 mM phosphate buffer (pH 6.2) containing autoclaved *Escherichia coli* cells and 250 μg/m*l* of streptomycin sulfate, as described previously (ITO and IWABUCHI, 1970). Ethanol was added to the liquid medium at the time of spore innoculation. The strain A3 cells which were cultured in HL5 medium (COCUCCI and SUSSMAN, 1970) were exposed to ethanol at various concentrations. The number of cells was counted with a hemacytometer.

Results

Effect of ethanol on spore germination

As Fig. 1 shows, spore germination was not affected with ethanol of less than 1%, but treatments with 1% and 2% ethanol made the proportion of germinated spores reduced by 90% and 69% that of the control, respectively. In the 2% ethanol treatment, emerged amoebae shrunk and could not proliferate. With 5% ethanol, the spore germination was completely inhibited, although almost all the spores could normally swell (71% of the control).

Effect of ethanol on growth of NC4 cells

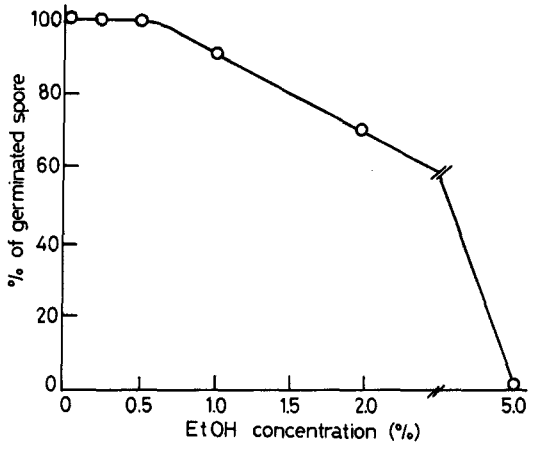

Fig. 1. Effect of ethanol on the spore germination. Spores of the strain NC4 were heat-shocked and incubated in a medium containing ethanol at 23°C. At 6 h after the onset of incubation the number of germinated spores was counted with a hemacytometer. The relative proportion of germinated spores in a ethanol-containing medium against those in the control medium is calculated and plotted in the figure.

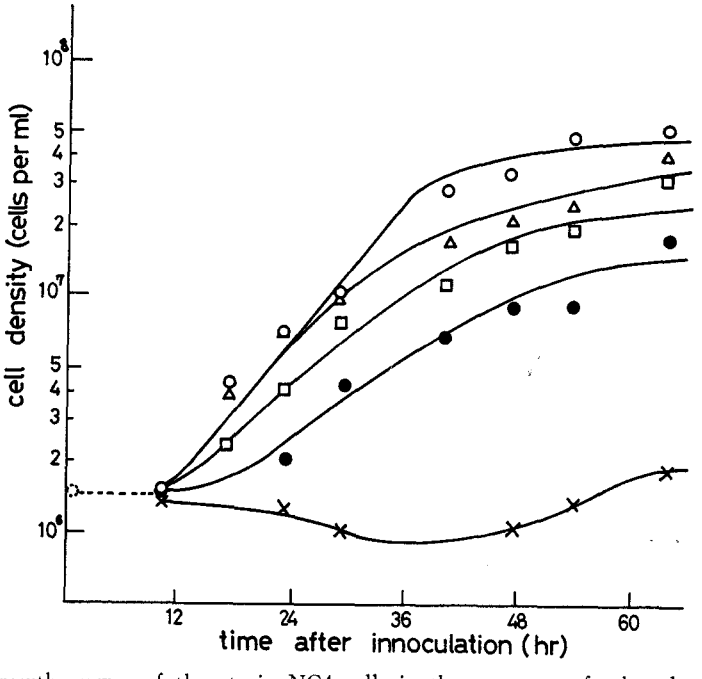

Fig. 2. Growth curves of the strain NC4 cells in the presence of ethanol. Spores of the strain NC4 were innoculated in the medium containing autoclaved bacterial cells. Ethanol was added to each culture, and the spores were incubated at 23°C in a shaker. Almost all the spores were germinated 10–15 h after the onset of incubation. A part of the medium was removed at the time indicated in the figure, and the cell number was counted with a hemacytometer. ○, control; △, 0.25% ethanol; □, 0.5% ethanol; ●, 1.0% ethanol; ×, 2.0% ethanol.

As shown in Fig. 2, the NC4 cells can grow in the presence of ethanol at the concentrations lower than 1%. Under the conditions, the cells appeared to be morphologically normal. However, the dose effect of ethanol on the cell growth was observed, that is, the average time of the cell generation was prolonged with an increase of the ethanol concentration (6.0 h for 0%, 7.0 h for 0.25%, 9.5 h for 0.5 % 12.0 h for 1.0%). In 2% ethanol treatment, the cells could proliferate only once after a long time of the lag-period. At this concentration of ethanol, the cells appeared shrunken and did not further grow.

Effect of ethanol on growth of A3 cells

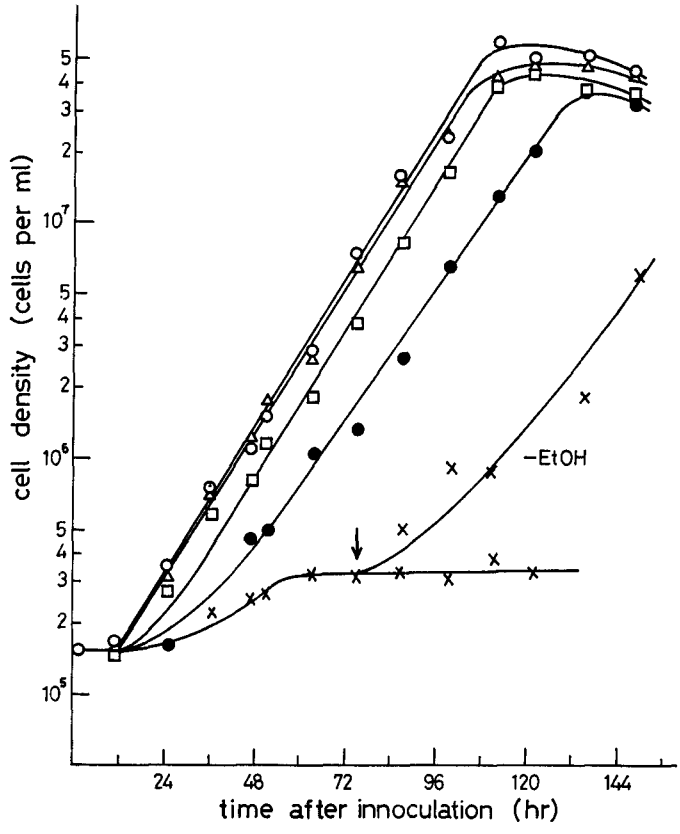

Fig. 3. Growth curves of the strain A3 cells in the presence of ethanol. One m*l* of the suspension of the A3 cells at the stational phase was added to 50-m*l* medium containing various concentrations of ethanol. After a lag-period of growth, the cells began to grow logarithmically. The number of cells were counted as in Fig. 2. The arrow indicates the removal of ethanol. ○, control; △, 0.25% ethanol; □, 0.5% ethanol; ●, 1.0% ethanol; ×, 2.0% ethanol.

The growth curves of the A3 cells at various concentrations of ethanol were somewhat different from those of the NC4 cells (Fig. 3). The time of a lag-period was dependent on the concentration of ethanol. When the concentration of ethanol was lower than 1%, the cells began to grow at almost normal rate after a definite time of a lag-period. Upon the treatment with 2% ethanol, however, the A3 cells proliferated only once but did not further grow. The morphological changes of the cells were similar to those of the NC4 cells exposed to ethanol.

When the cells which had been cultured for 72 h in the presence of 2% ethanol were transferred to the normal medium without ethanol, they began to grow, though at a slower rate, and thereafter their growth gradually returned to the normal rate (Fig. 3).

Discussion

The molecular effects of ethanol have been studied using mammalian cells (SCHEIG *et al.*, 1966; REITZ *et al.*, 1973; LITTLETON and JOHN, 1977). It is generally said that ethanol may produce a membrane perturbation to change the lipid composition and membrane properties. Recently, the effect of ethanol on the cell growth was studied in *Tetrahymena* by MANDINI-KISHORE *et al.* (1979), who showed that ethanol brought about a dose-dependent inhibitory effect on the cell growth of this organism. They have mentioned the following three points as the action of ethanol; the first is an increase of highly unsaturated fatty acids (18 : 2 and 18 : 3) and a decrease of palmitoleate (16 : 1) in cell membrane. The second is the compositional changes of phospholipids in the membrane. And, the third is that ethanol itself fluidizes the membrane. Thus, they have concluded that these ethanol-inducing changes resulted in the perturbation of the normal structure and function of cell membrane, so that the growth rate was reduced.

The molecular mechanism for the effect of ethanol on the growth of cells of *D. discoideum* strain NC4 is possibly explained in the light of the above idea proposed by MANDINI-KISHORE *et al.* However, it seems difficult to interpret the results of experiments with the A3 cells according to the same idea. The key to solve this problem may be hidden in the phenomenon of the dose-dependency of the ethanol-induced lag-period which was observed in the growth of the A3 cells. Probably, the A3 cells modified their membrane to adapt the environment with ethanol during this lag-period. What molecular events occur during this lag-period in the A3 cells is under the investigation.

The difference between the NC4 and A3 cells in the membrane pro-

perties seems to be reflected on their difference in the effect of ethanol on the cell growth. I can point out the following fact as evidence showing the difference between the two strains in the membrane properties; the A3 cells can uptake the nutrients from a bacteria-free medium by means of pinocytosis, while the NC4 cells feed the bacterial cells as nutrient without doing pinocytosis. The molecular mechanism for the action of ethanol on living cells should be investigated from this point of view.

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