invalid, the S phase (of an individual egg) could be as short as the time needed for the doubling of the largest replicating unit (approx. 4 min, Fig. 1 c). Our finding that 14% of the molecules are in replication is fully compatible with both of these extreme possibilities.

We thank Mrs. A. Poppitz and Mrs. I. Scholz for valuable technical assistence. Supported by DFG Mo 158/14.

Received May 12, 1980

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Cell Fusion Induced by High Electric Impulses Applied to *Dictyostelium*

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When cells of the eukaryotic microorganism *Dictyostelium discoideum* are repeatedly exposed to electric fields, they fuse into multinuclear, motile cells, the largest ones containing more than 40 nuclei. Under optimal conditions, i.e., initial field intensities of 4 to 6 kV/cm with decay times of about 40 µs, lysis of cells as a result of membrane permeabilization is negligible. This means that cells can be fused extensively without substantial loss.

The apparatus used for generating high electric impulses of short duration has been described elsewhere [1]. Most of our experiments were carried out using the *D. discoideum* strain Ax-2, which is adapted to growth in liquid medium [2]. However, similar results were obtained with a wild-type strain, v-12/M2, grown in suspension on *E. coli B/r* as a nutrient.

Impulses of the initial field intensity E_0 decaying exponentially with time according to $E(t)=E_0 \cdot \exp(-t/RC)$ were applied (R resistance of the cell suspension; C capacitance of the discharge circuit). Cells were suspended in 17 mM Soerensen phosphate buffer pH 6.0 at a density of 10^7 or $2 \cdot 10^8$ cells/ml and, in the latter case, bubbled with oxygen before transferring 1-ml samples into a chamber equipped with two flat platinum electrodes separated by a distance $d=1.4$ cm. At the lower density the cells had to be agglutinated by rolling the suspensions in plastic tubes in order to produce maximal contact between the cells before subjecting them to field impulses. Agglutination strongly increased the yield of fused cells.

Repeated impulses given at intervals of 1 to 3 s were more efficient than single ones. Our standard procedure consisted of 3 or 4 impulses applied at intervals of 3 s. Cell fusion was found to have occurred as soon as the cells could be removed from the electrode chamber and put under a microscope, i.e., within the first few seconds after the repeated exposure to an electric field.

$MgCl_2$ or $CaCl_2$ at a concentration of 2 mM inhibited fusion when present during field application. Since the absence of divalent cations resulted in delayed lysis of the cells as manifested at about 0.5 h after field application, the fused cells were diluted into phosphate buffer containing 2 mM $CaCl_2$.

When cells of strain Ax-2 were used, best results were obtained with impulses of an initial field intensity $E_0=5.7$ kV/cm. With a $R=1.8 \cdot 10^3\ \Omega$ and $C=21$ nF, the decay time constant was $RC=38$ µs. The temperature increase due to Joule heating was calculated as $\Delta T \simeq 0.18$ °C per pulse; hence the temperature increase appears to be too small for facilitating cell fusion. Quantitative data for a typical experiment are given in Fig. 1.

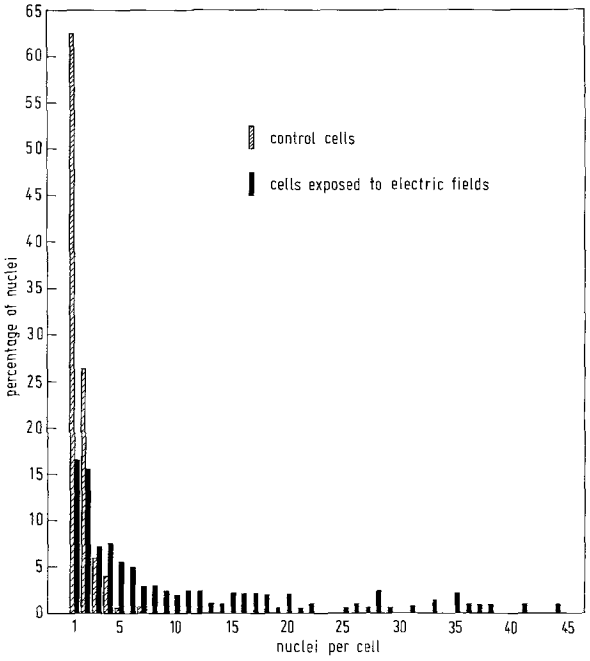

Fig. 1. Histogram showing the increased number of nuclei residing after electric field application in multinuclear cells. 35% of all nuclei were found in cells containing ⩾10 nuclei, and 9% in cells containing ⩾30 nuclei. Cells of these sizes were absent from the controls. In the experiment shown, cells of *D. discoideum* strain Ax-2 were grown in nutrient medium as described previously [7] and harvested during exponential growth. Cells were washed in 17 mM Soerensen phosphate buffer pH 6.0 and resuspended in the buffer at a density of 10^7 cells/ml. Fifteen minutes before field application the cells were allowed to agglutinate under gentle shaking in a roller tube. An electric field of $E_0=5.7$ kV/cm was applied three times at intervals of 3 s at 23 °C. Samples of 100 or 200 µl were transferred onto the Teflon membrane of Petriperm dishes (Heraeus, Hanau, Germany) in order to supply the cells with oxygen. Five min thereafter 3 ml of 2 mM $CaCl_2$ solution in phosphate buffer was added, and the cells were allowed to settle and spread on the Teflon surface. The cells were fixed on the surface in 70% ethanol. Nuclei were counted after staining with Mithramycin, a fluorescent stain for DNA [8] which brilliantly labeled nuclei and mitochondria

Field intensities of 7 kV/cm or higher caused lysis of many of the cells. Likewise, with a higher capacitance $C = 52$ nF and a resulting decay time constant of $RC = 104$ µs, a large fraction of the cells was lost because of substantial lysis, even when the initial field intensity was kept at $E_0 = 5.7$ kV/cm. Wild-type cells of the v-12/M2 strain were more sensitive than those of Ax-2. Consequently, the field intensity was reduced for v-12/M2 to 4.3 kV/cm and the number of impulses limited to 2 in order to avoid substantial lysis.

Electric fields may cause drastic permeability changes in the membranes of various cells and organelles [1, 3]. The primary effect of the field impulses is a transient and local perturbation of the structure of the membrane, which only slowly anneals after pulse termination [4]. In agglutinated cells of *D. discoideum* the plasma membranes are in contact with each other. Field-induced perturbations at sites of adhesion are apparently the starting points for the formation of larger cells by membrane fusion.

Cells of *D. discoideum* appear to contain no sialic acid at their surfaces, hence they are devoid of receptors for Sendai virus, one of the best fusing agents of mammalian cells [5]. Also, polyethylene glycol, now the most widely used fusing agent [6], did not, in our hands, produce cell fusion in *D. discoideum*. Thus, electric impulses provide the method of choice for cell fusion in this organism. The electric field method might be adaptable to fusion of other cells also.

In *D. discoideum* the method opens a possibility for mass production of heterokaryons. These can be used in genetic studies as a source of recombinants, as well as in biochemical investigations on the complementation between different mutant nuclei residing in a common cytoplasm. In this context it is of importance that giant multinuclear cells produced shortly after the end of growth are able to develop and to participate in aggregation.

We thank C.-R. Rabl for cooperation. Our work was supported by the Deutsche Forschungsgemeinschaft.

Received April 21, 1980

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Tumor Inhibition by Titanocene Dichloride: First Clues to the Mechanism of Action

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Metallocene dichlorides, $(C_5H_5)_2MCl_2$, exhibit cancerostatic activity against the Ehrlich ascites tumor (EAT) system in mice. Optimum activity leading to 100% cure rates is observed with M = Ti [1–3], V [4], Nb [5], and Mo [6]. The mechanism of action of this species of organometallic complexes is yet unknown.

Recently the directly cytostatic and cytotoxic effects of metallocene dichlorides have been demonstrated in vitro by determination of the cell growth inhibition and by vital staining [7]. In the present study we report on the influence of an in vivo treatment with titanocene dichloride on the following in vitro incorporation of ^{3}H-labelled, specific precursors of the DNA, RNA, or protein synthesis, respectively, into the acid-insoluble fraction of EAT cells.

About $6 \cdot 10^6$ EAT cells per animal were transplanted intraperitoneally (i.p.) on 60 female CF1 mice. Four days later the tumor was well developed. Now 30 animals received a single i.p. injection of 90 mg titanocene dichloride/kg, prepared and purified according to [8], and dissolved in 0.4 ml of a mixture of dimethylsulfoxide and saline (v/v = 1/9). Another 30 animals served as untreated controls and obtained an i.p. injection of 0.4 ml of only the dimethylsulfoxide-saline mixture. At various intervals up to 96 h after injection (Fig. 1) cells were removed in equal amounts (determination by use of an electronic particle counter Coulter Counter DN) each from 2 treated and 2 untreated animals and equally distributed to 3 samples per animal. Each sample contained 5 ml mini-

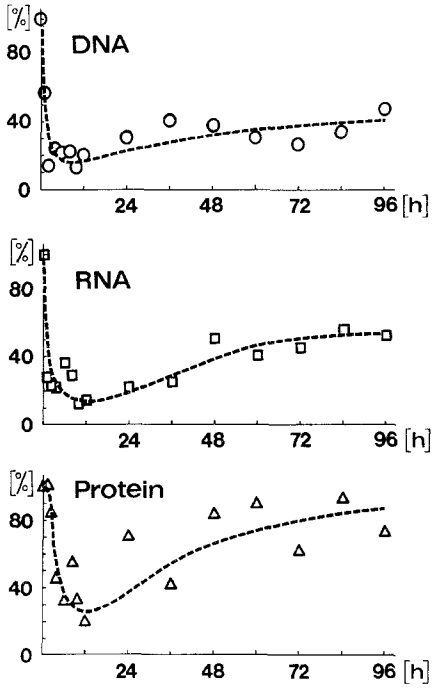

Fig. 1. Relative incorporation rates of [*methyl*-^{3}H]thymidine, [5-^{3}H]uridine, and L-[4,5-^{3}H]leucine as measures of the DNA, RNA, and protein syntheses in EAT cells. At 0 h in vivo treatment with 90 mg titanocene dichloride/kg. Cell harvesting after the residence times in vivo given on the abscissa, followed by an incorporation period of 20 min in vitro

