Interaction of DEAE-Dextran with Mammalian Cells Cultivated in vitro

DEAE-dextran (dimethylaminoethyldextran, hereinafter DEAE-D) is a cation polymer, possessing unusual properties in direct interaction with cells; it inhibits the formation of certain tumours in mice[1–3] and stimulates pinocytosis and incorporation of macromollecular substances into the host cell[4,5]. The faculty of increasing the uptake of substances by the cell has been attempted to be made use of also in our further work, continuing our experiments hitherto in which the incorporation of exogenous DNA into mammalian cells in vitro has been studied[6]. With regard to the fact that incorporation of this DNA takes place only in well proliferating cells, it was first necessary to ascertain whether DEAE-D does not inhibit the growth of mammalian cells cultivated in vitro, similarly to the results of experiments performed on tumour cells[1–3]. For this reason we focused our attention on the study of growth, morphological properties and karyotype of L-strain mouse fibroblasts after the application of DEAE-D.

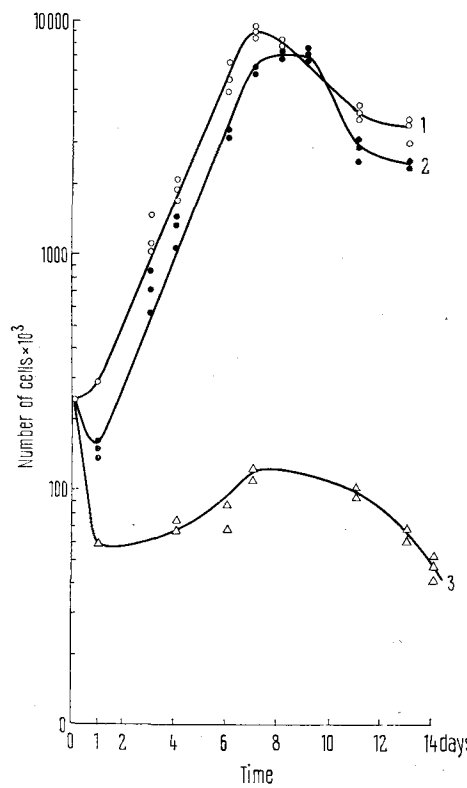
For the experiments, L-cells cultivated in monolayer in minimum Eagle's medium supplemented with 10% inactivated calf serum and antibiotics were used. DEAE-D (m.v. 2×10^6, Pharmacia Uppsala) was applied for periods of min, 30 or 24 h in the concentration of 500 µg/ml; as was found in our previous experiments, maximum incorporation of DNA occurs at this concentration[7]. After preincubation of the cells in DEAE-D, the monolayer was washed with DEAE-D-deficient medium, whereupon the cells were fixed or further cultivated in DEAE-D-deficient medium and then used for further experiments.

The results obtained showed that the degree of damage to the L-cells depended on the time for which the cells had been preincubated in DEAE-D; after a 30 min application, no morphological changes in the cells occurred in comparison with the control population; after 3 h, and especially after 24 h, of incubation of the cells in the DEAE-D-containing medium, the cell membrane displayed considerable damage, the cytoplasm was vacuolized and granulated, the nuclei were deformed, and the growth of the cells was stopped. A 30 min action of DEAE-D did not inhibit the growth of the cells, even though the cells entered the logarithmic phase later than the control cells (Figure). The length of the generation time (determined after 30 min action of DEAE-D on the basis of the increase of cells seeded in mitosis after synchronization of the population[8]) was approximately equally long (18.5 h) as in the controls (17.5 h). In the length of the G 2 phase and the S phase the cells, after preincubation with DEAE-D, differed from the controls (Table).

Short cultivation of cells in DEAE-D did not influence, either, the structure of the chromosomes; with the exception of a few quite isolated cases (2 chromosomes from 60 metaphases), no aberrant chromosomes were found in the set of chromosomes in the metaphase. Chromosome aberrations did not take place, even in the case when the cells were repeatedly cultivated always 30 min in the DEAE-D-containing medium and afterwards were transferred for 24 h into a DEAE-D-deficient medium. After preincubation of the cells in DEAE-D, the variation width of the number of chromosomes, however, changed and from the original modal number of 54 (range 50–58) occurred in the cell population with a greater variation width (range 45–60) and modals of 51 and 53 chromosomes (Table); endoreduplication, nor any other anomalies during mitosis were not noticed.

The results of the experiments show that, unlike transplantable tumour cells[1–3], short single application of DEAE-D (500 µg/ml) does not act on the growth of L-cells in an inhibitory manner. Of decisive importance here will, of course, be not only the type of cells, but primarily the concentration of DEAE-D and the duration of its action[4,10]. We believe that the inhibition of cell growth after the application of DEAE-D may be connected with the changes in the structure of the cellular membrane, especially of its electric charge and permeability[3]. These properties may be in tumour cells, to a certain degree, specific and different from non-tumorous cells[10]. At low doses of DEAE-D or its shorter action, an interaction occurs between the surface cell and the DEAE-D molecule, whereby the uptake of certain macromolecules is stimulated (e.g. albumin[11] or virus DNA[4]).

The results of our previous experiments have also shown that DEAE-dextran can increase the uptake of



Growth curve of L-cells after DEAE-dextran application. Control line without DEAE-D (1), cells after 30 min (2) or 3 h (3) application of 500 µg/ml DEAE-dextran.

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Influence of DEAE-D on the growth characteristics and the number of chromosomes in L-cells

Duration of action of DEAE-D (500 µg/ml)	Relative plating[a] (%)	Generation time (h)	S-phase[b] (h)	G2 phase[b] (h)	Mitotic index (%)	Number of chromosomes	
						Modal	Range
0	100	17.5	7.5	3.5	5.4	54	50–58
30 min	62–80	18.5	6.5	4.5	4.4	51 and 53	48–60

[a] Absolute plating efficiency 75–86%. [b] Determined by autographic technique in asynchronous population[9].

exogenous DNA in mammalian cells cultivated in vitro. For the experiments we used ^{3}H-DNA isolated from L-cells[11], as host cells L-cells cultivated in monolayer in minimal Eagle's medium supplemented with calf serum and carrier thymidine. After the application of DEAE-D, the incorporation of ^{3}H-DNA determined at several intervals by the specific activity of L-cell DNA[12], was 20 times higher than in the DEAE-D-deficient series[7].

The kinetics of the incorporation of exogenous DNA and its localization in the host cell in connection with the function and the mechanism of the effect of DEAE-D on the uptake of this DNA are the subject of further detailed studies.

Zusammenfassung. Wachstum, morphologische Eigenschaften und Karyotypie kultivierter L-Zellen wurden nach Applikation von DEAE-Dextran im Inkubationsmedium untersucht. In einer Konzentration von 500 µg/ml (was die Aufnahme von exogener DNS fördert) und bei einer Einwirkung während 30 min verändert DEAE-Dextran die untersuchten Eigenschaften nicht.

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In vitro Response of Human Leukocytes to Anti-Human-Thymocyte Globulin

Heterologous antilymphocyte serum and its globulin derivative (ALG) prolong homograft survival in vivo in man and animals[1–3]. However, there are variable and often conflicting reports on its mitogenic effects in vitro. Several laboratories[4–8] have reported its stimulatory effect on DNA synthesis, while others[9, 10] have reported its inhibitory effects on synthesis of DNA.

The present report shows that within a narrow dose range antilymphocyte globulin has a stimulatory effect on DNA synthesis by cultured human leukocytes, but if the concentration of ALG is increased, DNA synthesis is inhibited, at least partly because of the cytotoxic properties of these globulins for peripheral blood leukocytes.

Materials and Methods. The antilymphocyte serum (ALS) and ALG used in this study were made in a horse repeatedly immunized with human thymic cells[11]. The ALG, purified by DEAE-cellulose batch chromatography, contained a single IgG band detectable by immunoelectrophoretic techniques. Complement was removed from all antisera by heat inactivation at 56 °C for 30 min. The lymphocytotoxic titer of ALS was 1:6400 and of ALG, 1:3200, against human thymocytes in the presence of guinea-pig complement.

Blood samples were obtained from normal adults. The separation of leukocytes (by sedimentation method), leukocyte culture and determination of the degree of stimulation of DNA synthesis were done according to the methods used by PRASAD et al.[12]. Dilutions of NHS, ALS and ALG were made in Hank's balanced salt solution. Phytohemagglutinin M (PHA) was reconstituted with triple distilled water. The diluted sera and the undiluted PHA were added to the medium (0.1 ml per tube) prior to the addition of the cell suspension. 0.5×10^6 leukocytes were suspended in 2.5 ml of culture medium for each culture. Thymidine-methyl-H^3 (specific activity, 12 ci/m mole) was used to determine the degree of stimulation of DNA synthesis. 5 h prior to harvest 0.5 µci of the isotope was added to each culture tube. Experiments were repeated 3 times with similar results with leukocytes from 6 different individuals.

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