Effect of dibutyryl cAMP and theophylline on cultured rat embryonic shields

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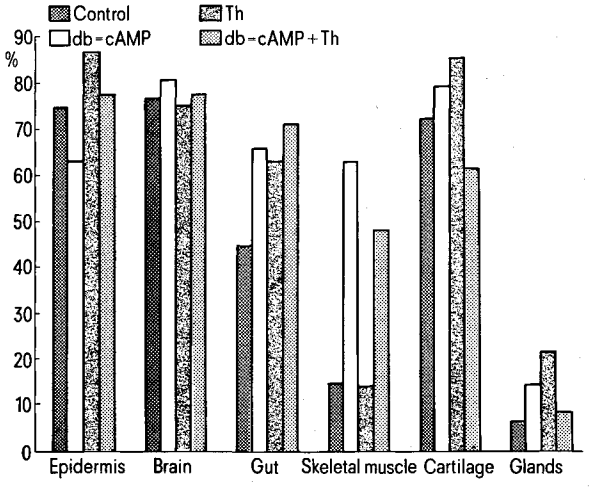
Summary. Effects of N^6, O^2 dibutyryl adenosine 3′,5′-cyclic monophosphate (db-cAMP) and theophylline (Th) on cultured rat embryonic shields were studied. After addition of db-cAMP to the culture medium, an increase of the weight of explants and of the incidence of the skeletal muscle was observed. Theophylline seems to be ineffective.

Cyclic nucleotides are known to control the growth of mammalian cells in cell culture[1] and the differentiation of different embryonic cells[2]. db-cAMP can stimulate the cell growth and accelerate the appearence of gamma-crystallins in the monolayer culture of rat lens epithelial cells[3]. In mouse neuroblastoma cells in vitro, the same cyclic nucleotide irreversibly induces several differentiated functions characteristic of mature neurons[4]. Finally a neuralizing influence of db-cAMP on the undetermined amphibian[5] or chick[6] ectoderm has recently been observed. The modified organ culture of rat embryonic shields was shown to provide favourable conditions necessary for the differentiation of main tissues[7]. However, the histological differentiation in explants proved inferior to that obtained in homografts of the same shields under the kidney capsule. The purpose of the present experiment was to find out whether the addition of db-cAMP and/or Th to the culture medium can improve the phenotypic expression of whole rat embryonic shields as described above for different vertebrate cells in vitro.

Materials and methods. Female rats of the inbred Fischer strain were killed after 9 days of pregnancy and the egg-cylinders at the primitive streak stage were isolated. The extraembryonic part was cut off at the level of the amnion and the shields were put on the lens paper supported by a stainless steel grid placed in an embryological watch glass as described previously[7]. The liquid medium consisted of Eagle's minimum essential medium supplemented with 40% of rat serum. From 5 to 14 days db-cAMP (Sigma), Th or both agents together were added to the medium in the concentration of 10^{-3} M. After 14 days the explants were fixed, and histological sections were examined.



Incidence of tissues found in explants of rat embryonic shields after 2 weeks of culture. Control series = 112 explants; 10^{-3} M db-cAMP = 62 explants; 10^{-3} M theophylline = 63 explants; db-cAMP and Th = 77 explants.

Results and discussion. From the figure one can see that the gut epithelium appears more frequently in the treated series than in controls ($\chi^2 = 7.4$; $p < 0.01$). However, before the evaluation of this result, previous data have to be taken into consideration. In all untreated series, obtained so far (from 350 explants) the percentage of the gut epithelium was the same as in the series treated with db-cAMP (60%). This result must therefore be interpreted with great caution, in spite of the fact that we cultivated parallel series with and without db-cAMP. On the other hand the skeletal muscle, which also appears more frequently in the treated series than in controls ($\chi^2 = 40.65$; $p < 0.001$), seems to reflect the real situation. Our previous experiments are in full agreement with recent control series. The skeletal muscle is always rare, its incidence never exceeding 20% (in 400 explants). Butyric acid either had a toxic effect (10^{-2} M) or had no effect whatsoever (10^{-3} M). For the time being it is difficult to give any plausible explanation for this action of db-cAMP on the appearance of the skeletal muscle. Theophylline, which is known to increase the intracellular pool of cyclic AMP through phosphodiesterase inhibition, usually acts in the same way as the addition of db-cAMP, while in our system Th seems to have been ineffective. However, results similar to ours have been obtained in different systems when Th had no visible effect[3,6]. Furthermore, db-cAMP and Th proved to inhibit the cell growth and the fusion of mononucleated myoblasts into multinucleated myotubes in a monolayer culture[8]. In our experiment, we obtained just the opposite effect. The percentage of myotubes in explants after addition of db-cAMP was higher than in controls.

Similar results were recently published and it was claimed that, under the proper conditions, experimental elevation in cAMP can in fact stimulate myoblast fusion[9].

During our experiments, we observed that the surface of the histological sections of treated series was larger than that in controls. Therefore we weighed some explants before fixation. The weight of the control series was 0.217 ± 0.25 g (n = 29), and in the presence of db-cAMP: 0.401 ± 0.022 g (n = 27). The difference is statistically highly significant ($t = 5.79$; $p < 0.001$). In our previous experiments we had observed that, during the first week of culture, the growth of explants was clearly visible,

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while in the second week the explants gradually became smaller. When measuring the amount of DNA, RNA and protein, the curve indicated the same decline during the second week (unpublished results). The action of db-cAMP might therefore be explained by the inhibition of cell death/or the stimulation of cell proliferation. We must bear in mind that the growth-promoting effects of cAMP are sometimes claimed to be due to the restoration of the purine nucleotide pool and not to a specific influence[10].

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Differences in cytochalasin D-induced surface alterations between chronic lymphocytic leukaemic and normal lymphocytes[1]

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Summary. Cytochalasin D (CD) causes an unusual surface alteration in normal lymphocytes consisting of the formation of focal irregular club-shaped cell processes. Lymphocytes from chronic lymphocytic leukaemic cases did not show this change on exposure to CD. There was either no surface change or, in some cases, clear double-membrane lined vesicles were formed and appeared to be discharged from the cell. This difference in response may be related to the changes in cell membranes known to occur in malignant transformation.

The effect of cytochalasins on mammalian cells are numerous and are thought to be brought about by an alteration of the cell microfilament function[2–5]. Studies with tritiated cytochalasin D (CD) support this concept as it is taken up and bound to the subplasmalemmal microfilaments or cell membrane[6]. Previous ultrastructural study on the effects of CD on the morphology of cultured tumour cells showed cytoplasmic projections thought to be similar to zeiotic blebs[7]. The present study of the effects of CD on normal human peripheral blood lymphocytes show the morphological surface alterations to consist of unusual cytoplasmic processes. These processes were focal and did not undergo repeated protrusion and retraction in contrast to the phenomenon of zeiosis[8]. The change seen was in keeping with the concept of the action of the cytochalasins on the microfilaments. In the cases of chronic lymphocytic leukaemia (CLL) there was either no marked surface change or, in a few cases, there was formation of double membrane lined vesicles at the cell surface. The reason for the different morphological response in the chronic lymphocytic leukaemic lymphocytes was not apparent from this study.

Materials and methods. CD was dissolved in dimethylsulfoxide (DMSO). Lymphocytes were obtained from normal donors and from cases of CLL. The following lymphocyte preparations were used. 1. Lymphocytes in leucocyte-rich plasma without further manipulation, 2. lymphocytes separated on a Ficoll-Hypaque gradient, washed and suspended in growth medium (GM) consisting of Eagle's medium with 10% fetal calf serum, 3. lymphocytes separated on a Ficoll-Hypaque gradient, washed with GM and resuspended in plasma. Cell suspensions at a concentration of 1 million cells per ml were incubated for 40 min at 37 °C with a final concentration of 1. CD, 1 µg/ml and 2. CD, 15 µg/ml. The concentration of DMSO was 0.5%. Controls with and without 0.5% DMSO were also prepared. Unfixed wet preparations were studied immediately at the end of the 40 min incubation by phase microscopy. The cell suspensions were then fixed in ice-cold 2% gluteraldehyde for scanning electron

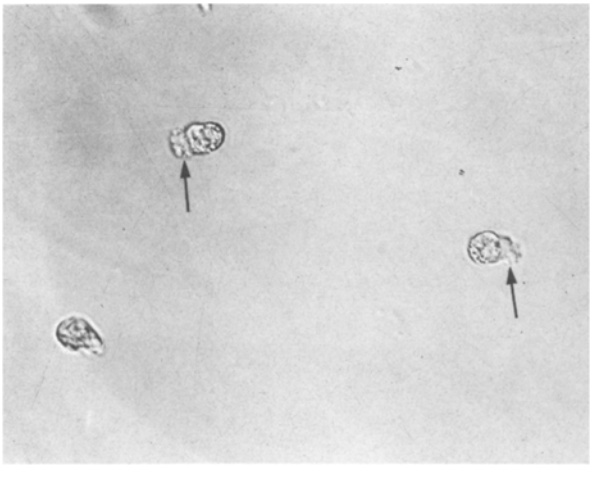

Fig. 1. Arrows point to focal cytoplasmic irregularities at 1 pole of normal lymphocytes exposed to CD 15 µg/ml, unfixed wet specimen. ×550.

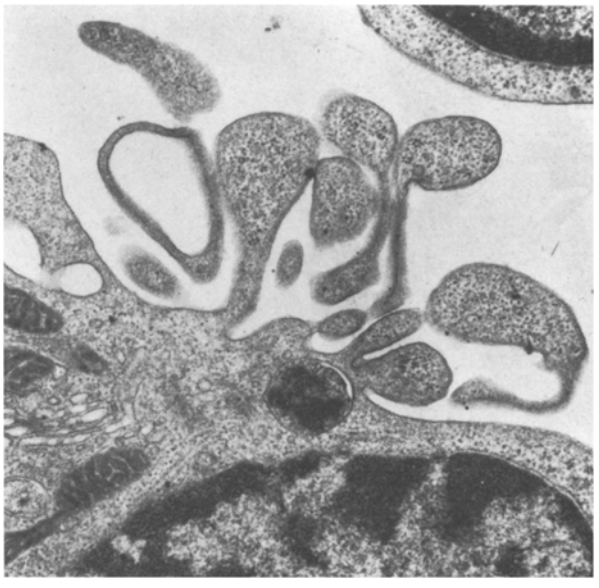

Fig. 2. TEM of above preparation showing surface irregularities to be cytoplasmic, club-shaped processes. × 21,000.