Effects of cytochalasin H on the embryonic endodermal cells of Microhyla ornata

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Summary. Cytochalasin H (CH) like CB causes disaggregation of embryonic endodermal cells and reduces their adhesivity to the glass surface. These cells reaggregate on removal of the drug from the medium. Reversibility depends on the duration of drug treatment. The mechanism of drug action is explained.

Cytochalasin B (CB), a fungal metabolite, has been shown to affect a number of biological processes. It is known to inhibit cytokinesis[2-4], cell movement[2,5-7], mucopolysaccharide synthesis[8], to bring about enucleation[9,10], and disaggregation of embryonic cells[11].

Cytochalasin H (CH) has recently been isolated from the fungus Phomopsis paspali by Pendse[12]. Structurally CH is different from CB in that CB belongs to the group of 24 oXa (14) cytochalasins while CH belongs to a different structural Sub-group 11 cytochalasan. CH is similar to cytochalasin D (CD). Biological effects of CH have been studied in our laboratory on a variety of organisms. It is found that CH like CB causes disaggregation of embryonic cells. Embryonic endodermal cells of the frog Microhyla ornata are used in studying its effects, and observations in understanding the mechanism of its action are communicated in the present note.

Materials and methods. Freshly laid eggs of the frog Microhyla ornata were collected from natural ponds and allowed to develop further in HF/10 medium till early gastrula stage (Dorsal lip Crescent) was obtained. The eggs were then dejellied and vitelline membrane was removed. These eggs were then transferred to a medium (HF/10) containing CH (1.5 µg/ml). The pH of the medium was found to be 6.7. Equal number of eggs were kept in HF/10 containing DMSO without CH. These served as controls. Stock solution of cytochalasin H (CH) was prepared by dissolving 1.0 mg of compound in 1.0 ml of dimethyl sulfoxide (DMSO) and was stored in the deep freeze compartment of the refrigerator. From this solution, fresh working solution having 1.5 µg/ml concentration was prepared in Holtfreter's solution (HF/10).

Disaggregation of cells started within 25 to 30 min of the treatment of CH. The endodermal cells were the first to disaggregate. Within 1.5 h almost all the endodermal cells were loose, forming an unorganised heap of cells. The control embryos developed normally and no disaggregation was caused. To test the reversibility of the action of CH on endodermal cells, the duration of exposure to CH was extended up to 2.5 h. Endodermal cells were pipetted out and washed 3–4 times in fresh HF/10 medium without CH and finally placed in a Maximov depression slide containing HF/10 medium. The Maximov slide was shaken occasionally in a gyratory motion to facilitate cell to cell collisions and eventual aggregation. Since the endodermal cells are incapable of active locomotion, such a gyratory motion was felt necessary. The cells were observed at definite intervals and photographed. The experiments were done in 3 different sets.

Results and discussion. It was observed that loose endodermal cells in the medium containing CH do not stick to glass surface readily and could be pipetted out easily. However, the cells when transferred to medium without CH show tendency to stick to glass as well as to other cells with considerable rapidity. At times, during the washing procedure, many cells are lost because they are stuck to the glass bottom of the Maximov slide or to the wall of the pipette, and only cells which were not stuck could be kept under observation for their aggregation capacity. It therefore seems that CH modifies or in some way alters the cell surface of the disaggregated cells, and when these cells are brought back into medium without CH, cell surface recovers to the original condition. The time required for the recovery of these cells varies, depending on the degree of the cell surface modification. The cells which were less affected showed quick recovery and rapid adhesivity to glass, while those which were affected more, required longer time for the recovery. Adhesivity of the Ehrlich ascites cells to glass was shown to be reduced on CB treatment[13].

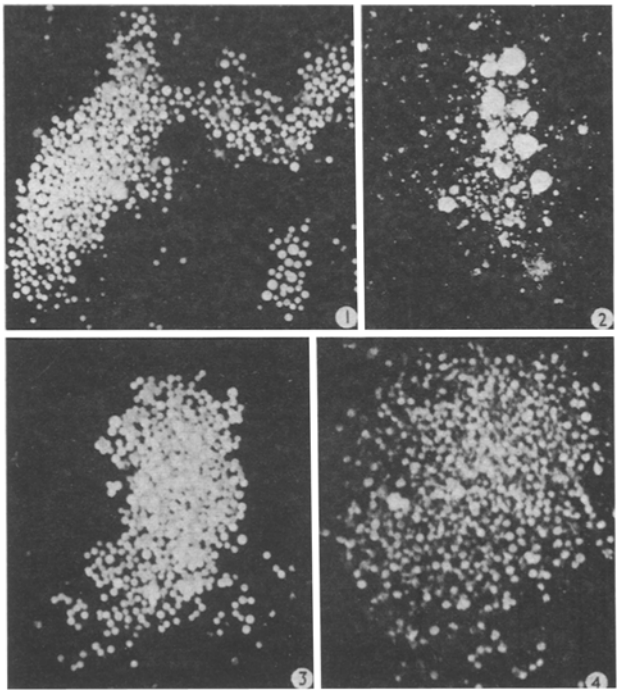

Fig. 1. Endodermal cells disaggregated in CH for 1 h 40 min kept for aggregation. ×24. Fig. 2. Same after 30 h. ×24. Fig. 3. Endodermal cells disaggregated in CH for 2.5 h and kept for aggregation. ×24. Fig. 4. The same after 24 h. ×24.

It is found that the loose endodermal cells (figure 1) when brought together could form small aggregates within 4–5 h and these aggregates grew in size, incorporating more and more cells in 24 h (figure 2). Thus showing that the effect of CH is reversible. Reversibility of CB effect on a variety of cells has been shown[2,6,14]. The reversibility depends on the duration of the treatment. If the exposure to CH was prolonged to 2.5 h, the disaggregated cells (figure 3) neither showed stickiness to glass nor did they form aggregates (figure 4). Such loose cells showed tendency to degenerate after 48 h. By 2.5 h exposure to CH, the cell surface of these cells was probably modified beyond recovery.

The action of CB, especially on cytokinesis and cell movement, is attributed to the disruption of the contractile

microfilament machinery[3,4,14–17]. However, it is difficult to explain its property of disaggregating embryonic cells on the above hypothesis. Experiments by Sanger and Holtzer[8] have shown the action of CB on the cell surface material which holds the cells together, while Schaeffer et al.[11] have proposed an alteration of cell surface charge causing disaggregation of embryonic cells. Our observations of the behaviour of endodermal cells, with and without CH treatment, the rapid recovery of some of the cells which stick to glass surface suggests the possibility that CH like CB acts by bringing about the alteration of cell surface charge as suggested by Schaeffer et al.[11]. However, the possibility of the inhibition of the synthesis of mucopolysaccharides by CH, as reported for CB by Sanger and Holtzer[8], cannot be excluded.

1 We thank the University Grants Commission India for financia assistance for the Research project and Mr J. C. Daniel the Curator of Bombay Natural History Museum for identification of the frog.

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Contractile filaments in cells of regenerating tendon[1]

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Summary. An extensive cytoplasmic fibrillar system has been observed in fibroblast-like cells of regenerating tendon. It consists of bundles of actin filaments, which often show a cross-striated appearance due to electron dense bodies occurring throughout their length. The functional role of this contractile apparatus seems to be related to the process of movement and orientation of the newly formed cells and to the retraction of the regenerating tendon.

Recent ultrastructural and immunofluorescent studies[3–7] have allowed the distinction between 2 different types of fibroblasts, namely a) the typical fibroblast, containing few randomly dispersed cytoplasmic filaments, probably endowed with contractile activity, and b) the so-called myofibroblast, which is provided with an extensive fibrillar system made up of bundles of parallel actin filaments. This latter type of cell, which can be considered as intermediate between typical fibroblast and smooth muscle cell, has been observed in the granulation tissue of healing wounds[4,5,7], in the chicken aorta[8], in the rat ovary[9], in the palmar nodules of Dupuytren disease[6], and in the tenocytes of newborn rabbit calcaneal tendon[10]. In the current study, evidence is provided for the presence of large amounts of actin arranged in bundles of filaments in the cytoplasm of regenerating cells during the early stages of morphological recovery of tendon.

Material and methods. Adult male New Zealand rabbits were used in the experiments. Under general anaesthesia and aseptic conditions, the right calcaneal tendon was exposed through a short incision of the skin and peritendineous tissues. The tendon was then cut transversely with a scalpel. A gap resulted between the 2 ends of the severed tendon due to retraction of the proximal stump, attracted by the triceps surae muscle; 7 days after operation the newly formed tissue bridging the gap was removed. The controlateral tendon was used as control. For the morphological study, the newly formed tissue and the control tendon were fixed for 10 min with 3% paraformaldehyde – 1% glutaraldehyde in Millonig buffer pH 7.4 by an 'in vivo' dripping method. The tissue was then removed, trimmed into small pieces, placed in the same fixative for 2 h and postfixed in 1% OsO_4. Specimens were dehydrated and embedded in Epon. Light microscopic observations were performed on 1 μm thick sections stained with buffered Toluidine blue (pH 8) or with buffered Blue de Unna (pH 7). For the ultrastructural study, thin sections were stained with lead hydroxide and uranil acetate, and examined in a Siemens Elmiskop 101 electron microscope.

1 Work was supported by grant C. N. R. n. 75.01224.04–115.5479 from Consiglio Nazionale delle Ricerche, Italy.
2 The authors are grateful to Dr G. F. Bottazzo, Dept. Immunology, Middlesex Hospital, London, for carrying out the absorption experiments with purified actin and to Miss M. Rita Nicotra, Miss Laura M. Vassallo and Mr Mario Termine for their technical assistance.
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