The Action of *Staphylococcus* α-Toxin on Cell Cultures: An Interference-Contrast Microscope Study

Staphylococcus α-toxin (α-haemolysin) is active against a great variety of cultures of tissues and cells in vitro[1–4]. α-toxin rapidly causes vacuolar degeneration, cytoplasmic coarctation and death of the cells in variable length of time, depending on the cell strain used[3].

The hypothesis has been put forward that α-toxin acts by altering the permeability of the cell membrane[5]. Actually, the toxin causes the lysis of lysosomes isolated from rabbit leukocytes, and induces the release of beta-glycuronidase and acid phosphatase from rabbit liver lysosomes in vitro[6]. Moreover, it has been demonstrated that the toxin interreacts, by means of a compound different from the toxin itself, with rabbit erythrocyte membranes and also with artificial membranes[7].

On the other hand, from the biochemical viewpoint, it has recently been demonstrated that α-toxin causes a rapid fall of intracellular levels of ATP in cells cultured in vitro[8]. Such a decrease is probably due to the uncoupling of oxidative phosphorylation and to a marked stimulation of Mg^{++}-dependent ATP-ase activity induced by the toxin[9]. The damage of the cell membranes by *Staphylococcus* α-toxin has been demonstrated in experimental models or in isolated sub-cellular particles that represent an experimental substrate different from the whole cell[6,7].

The interference-contrast microscope is an extension of differential interference microscope[10] yielding at the same time a good resolution, a good contrast and allowing the observation of the cell surface.

It therefore appeared of interest to study the cell surface modifications induced by α-toxin in cell cultures using an interference-contrast microscope in order to obtain further informations about the mechanism of action of the toxin itself.

Materials and methods. Cell cultures. HEp-2 cells (from human laryngeal carcinoma, supplied by A.T.C.C.[11], were cultured at 37°C in Eagle medium (Difco) supplemented with 10% inactivated calf serum, in Leighton tubes containing a coverslip. This cell strain is highly susceptible to the action of α-toxin[12].

Staphylococcus α-toxin. Crude *Staphylococcus* α-toxin (kindly supplied by Prof. A. DE BARBIERI, Istituto Sieroterapico Milanese, Milan, Italy) obtained from Wood 46 strain of *Staphylococcus aureus* and containing 1700 MHD/ml[13] was used. This toxin was exhaustively dyalized, before use, against Hanks' BSS to obtain the concentration of 85 MHD/ml. In previous experiments carried out by one of us, this toxin's concentration induced a cytopathic effect and a marked decrease of ATP cell levels within 120 min of contact with cell cultures[8].

Antiserum. Specific horse antiserum (Burroughs Wellcome) containing 150 IU/ml[14] was used.

Experiments. The nutrient medium was eliminated from the Leighton tubes and the cell cultures were washed twice with Hanks' BSS at 37°C. The medium was then substituted with 1 ml of *Staphylococcus* α-toxin at the previously indicated concentration. The cell cultures were therefore placed again at 37°C. After 30 min, 1 h, 3h and 6 h of incubation the coverslips were extracted from the Leighton tubes, washed twice with Hanks' BSS, fixed in glutaraldehyde and stained as reported eleswhere[15]. The specimens were then observed and photographed with a Leitz Orthoplan interference-contrast microscope. As control, in some cell cultures the nutrient medium was substituted by plain Hank's BSS or by α-toxin mixed to a neutralizing amount of specific antiserum.

Results. The control cell cultures, under the interference-contrast microscope, show a typical appearence. The nuclear membranes, the nucleoli and the chromosomes of the dividing cells are easily observable (Figure 1). In cell cultures subjected to the action of *Stapylococcus* α-toxin, after 30 min of incubation, some changes can already be detected. These consist of the appearance of small depressions, of the shape of craters and of variable number, that appear on the call surface and that become more severe after 1 h of incubation (Figure 2). After 3 h of incubation many cells show an almost complete breakdown of the membrane and in many cases inter-cellular contact is lacking (Figure 3). This cell damage, both in the initial stages and after 3 h of contact with alpha-toxin, is not common to all cells of the same culture, since almost normal cells, next to other seriously damaged cells, can be observed. After 6 h of incubation, all the cells undergo a complete lysis. The cell cultures treated with plain Hanks' BSS or with toxin-antitoxin mixture have always displayed a normal appearence.

Discussion. The findings reported in the present paper show early morphological modifications of the surface of the cells subjected to the action of *Staphylococcus* α-toxin. These alterations might be due either to a direct action of the toxin on the cell membrane, as happens with membranes of the lysosomes in vitro, either to alteration of the

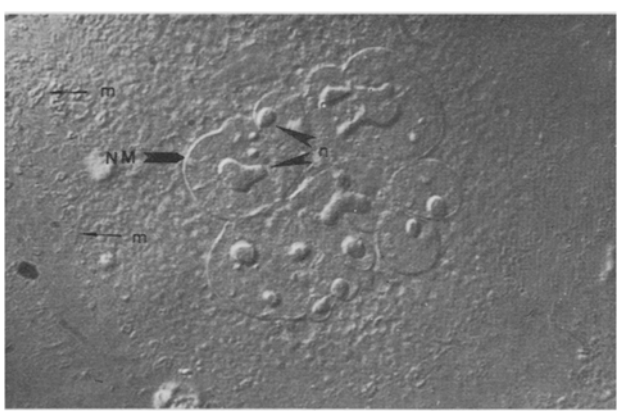

Fig. 1. Normal HEp-2 cell culture. A giant, multinucleated cell. The nuclear membrena (NM), the nucleoli (n) and some mitochondria are visible. ×1000.

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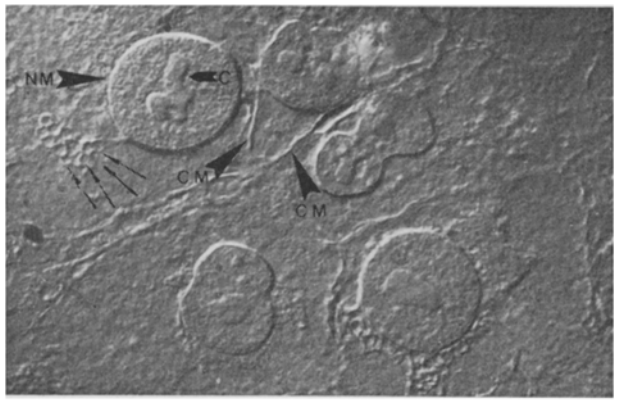

Fig. 2. HEp-2 cell culture after 60 min of contact with *Staphylococcus* α-toxin. The initial breakdown of cell membrane (CM) and numerous small depressions on the cell surface (arrows) are visible. NM = Nuclear membrane; C = chromatin. ×1000.

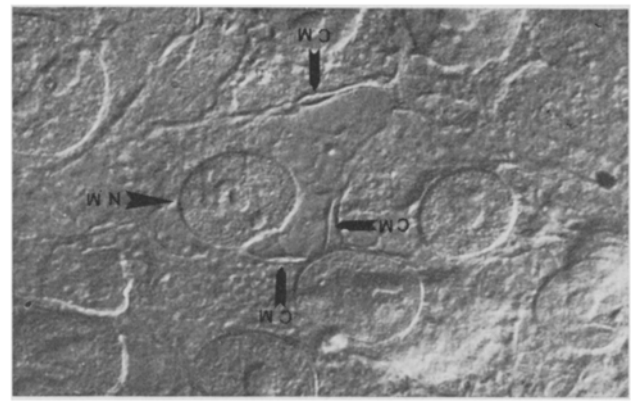

Fig. 3. HEp-2 cell culture after 180 min of contact with *Staphylococcus* α-toxin. A remarkable breakdown of cell membrane (CM) is present. The nuclear membrane (NM) and the nucleoli are barely visible. ×1000.

energetic metabolism, with a drop in the intracellular levels of ATP. This would call attention to what takes place in hemolytic anaemia associated with a congenital defect of glucose-6-phosphate-dehydrogenase activity, in which the fragility of erythrocyte membranes is caused, in part, by a decrease of the cellular content in ATP[16]. The changes of the morphology of the cell membrane described in the present paper take place with the same concentration of toxin and within the same times of incubation which cause the rapid decrease of intracellular levels of ATP[8]. On the other hand, the alterations of the membranes appear in stages notably different in different cells of the same culture. This behaviour may be due to the sensitive biochemical differences that can be checked among cellular populations[17–19]. The different stages of appearence of the alterations of the cell membranes induced by α-toxin in cells of the same culture could, therefore, make us believe that such alterations are caused by a lesion of the energetic metabolism rather than due to a direct action of the α-toxin on the membranes themselves.

Riassunto. Gli Autori hanno preso in esame, mediante il microscopio a contrasto di fase interferenziale, le modificazioni della superficie cellulare indotte dalla tossina alfa-stafilococcica in colture di cellule HEp-2. La tossina causa rapidamente collasso della membrana, seguito da rottura della membrana stessa. Vengono discussi i rapporti fra questi aspetti morfologici e le lesioni biochimiche indotte dalla tossina.

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Nucleolar Ultrastructure in the Quiescent Embryonic Cells of the Dry Seed of *Allium cepa* L.

Continuity of the nucleolus during the cell cycle is maintained by the nucleolar organizing region of the chromosomes. The recent clarification of the nucleolar chromatin, as the nucleolar organizer and the ribosomal cistron[1,2], has indeed emphasized the importance of the nucleolus in the area of protein synthesis. Nucleolar activity and its ultrastructural morphology may vary to a great extent depending on the physiology and specialization of cells[3]. Such changes also occur due to actions of various exogenously administered inhibitors[4] or promoters[5] of macromolecular synthesis. Even at the optical microscope level, reflections of physiological events on the nucleolar morphology are easily detected[6,7].

Our recent interest in quiescence[8,9] has led us to look at the nucleoli of the quiescent root meristem of *Allium cepa* L. bulb as well as the embryonic cells of its dry seeds. Differentiation observed in the nucleolar morphology are reported in this note.

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