CYCLIC CHANGES IN THE CELL SURFACE

II. The Effect of Cytochalasin B on the Surface Morphology of Synchronized Chinese Hamster Ovary Cells

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ABSTRACT

The surface morphology of Chinese hamster ovary cells treated with cytochalasin B (CB) has been examined using the scanning electron microscope. The cells respond to treatment with CB by retracting peripheral processes, rounding up, and assuming a smooth or gently convoluted surface. This response occurs within minutes. Cells in different stages of the cell cycle all respond in a similar manner. When CB is removed from treated cells by washing with conditioned medium, the cells regain their normal surface conformation within minutes. The surface topography of these released cells is characteristic of their stage in the cell cycle. Because CB causes an alteration in the morphology of the cell surface and because of the speed of the response and recovery, it is proposed that the primary site of action of CB is the cell surface.

INTRODUCTION

Cytochalasin B (CB) has many interesting effects on cellular morphology and on diverse other cellular activities. Early reports emphasized its disruptive effect on microfilaments (Wessells et al., 1971), but recently many workers have suggested that the primary site of action is the cell surface membrane. (*See* Discussion in preceding paper, Everhart and Rubin, 1974.) In the preceding paper we demonstrated that CB applied to cells at certain times in the cell cycle can block the normal acquisition of the capacity to transport thymidine. It has previously been demonstrated (Porter et al., 1973; Rubin and Everhart, 1973) that specific structural changes occur in the cell surface as cells progress through the cell cycle. It was therefore of interest to study the morphological effects of CB as related to the cell cycle stages. We have taken advantage of recent improvements in methods of preparation of samples for the scanning electron microscope. The improved resolution that resulted has allowed new insight into the interaction of CB with the cell surface.

MATERIALS AND METHODS

Chinese hamster ovary (CHO) cells were grown in Ham's F-12 medium supplemented with 10% fetal calf serum. The maintenance of stock cultures and growth of cells on cover slips were as described in the preceding paper (Everhart and Rubin, 1974). Preparations of mitotic cells were obtained by the shake-off technique described by Tobey et al. (1967). These cells were plated onto cover slips at low density and allowed to grow for several hours, after which time no cells had entered S. These preparations possessed the typical G_1 morphology described by Porter et al.

(1973). Cells in S and G_2 were obtained by the isoleucine$^{\ominus}$ synchronization procedure of Tobey and Ley (1971) and modified for monolayer cultures by Everhart (1972).

CB was obtained from Imperial Chemicals Industries, Ltd., Cheshire, England. Stock solutions were prepared in dimethyl sulfoxide (DMSO) at a concentration of 1 mg/ml. At the concentrations of CB used (1–10 μg/ml), this concentration of DMSO had no observable effect on the surface morphology of CHO cells as studied in the scanning electron microscope.

Cells were released from CB treatment by washing twice with conditioned medium obtained from replicate cover slip cultures grown under identical conditions but lacking CB.

Cover slip cultures of cells were prepared for scanning electron microscopy by the procedures described by Porter et al. (1972). Briefly, this involved fixation in glutaraldehyde, critical point drying from acetone, and coating with carbon and gold.

RESULTS

We have studied the effects of CB at various concentrations and different times of treatment on Chinese hamster ovary cells possessing two different morphologies. Cells initially plated out at very low densities in G_1 possessed a rounded, highly blebbed morphology as seen in Fig. 1 and as previously reported (Rubin and Everhart, 1973). When these cells were treated with 5 μg/ml of CB, the blebs began to disappear within 5 min. At the end of 15–20 min, most of the surface had become gently convoluted (Fig. 2). Concurrent with this change was the appearance of small groups (usually localized toward the periphery) of very small, irregularly shaped blebs (Fig. 3). Treatment beyond 30 min produced little noticeable added effect (Fig. 4). When the drug was removed by washing with conditioned medium, the cells began to return to their original, blebbed morphology within 2.5 min (Fig. 5). After 10 min, many cells were indistinguishable from controls although 45 min is usually required for all cells to have regained their fully blebbed configurations (Fig. 6).

Cells synchronized in S and G_2 by isoleucine deprivation are generally flat (Fig. 7), and therefore, show more pronounced shape changes under the influence of CB than G_1 cells. Within 5 min the microvilli of these cells disappear. After 10 min a pronounced retraction of the peripheral cellular processes is observed. The resulting morphology is shown in Fig. 8. At this time a convoluted topography of the cell surface has also become apparent. After 20 min the drug has generally achieved its maximal effect; the cells are oblong or rounded with many long extensions leading to the periphery of the original cellular margins (Fig. 9). The complete loss of normal microvilli and the occasional appearance of irregular bleb like extensions (different from those on normal G_1 cells, Fig. 1) are evident at this time. Little deviation is noted from this morphology for cells examined at 1, 2, and 3 h. When higher concentrations of CB are used (up to 20 μg/ml) the effects are identical, but the time-course is more rapid with maximal effect occurring between 10 and 15 min (Fig. 10). When the concentration is reduced to 1 μg/ml, no morphological change was seen after as much as 2 h.

When the CB is removed from S and G_2 cells treated with 5 μg/ml CB, a distinct alteration can be noted within 5 min. The cells begin to spread out and flatten onto the surface (Fig. 11). 10 min after release the surface topography of S and G_1 cells can be recognized, and after 15–20 min the cells are almost indistinguishable from untreated controls (Fig. 12). The appearance of microvilli, some blebbing over the central regions of the cell, and a highly flattened overall morphology are observed in the majority of these cells.

Irrespective of the original cellular morphology, cells always undergo the same general sequence of events after application and then removal of CB at 5 μg/ml. The cell surface changes occur immediately after the addition of CB and before gross changes in cell shape. On addition of the drug, all normal cellular surface processes retract and disappear. These reductions are first observed as soon as 2 min after CB application. Retraction is rapidly followed (within 10 min) by a convolution of the surface and the appearance of irregular blebs. General retraction of the cellular margins is observed after the initial surface changes have occurred. On removal of the drug these events are repeated in the reverse sequence, except that again the surface responds (i.e., it returns to its normal G_1, S, or G_2 configuration) more rapidly than the overall cellular morphology.

DISCUSSION

We have observed two separate responses of cells to CB treatment. The first is a rapid response of the cell topography, i.e., the fine structure of the cell surface. Cells respond by losing blebs and microvilli and taking on a coated appearance. The second response takes more time and involves the

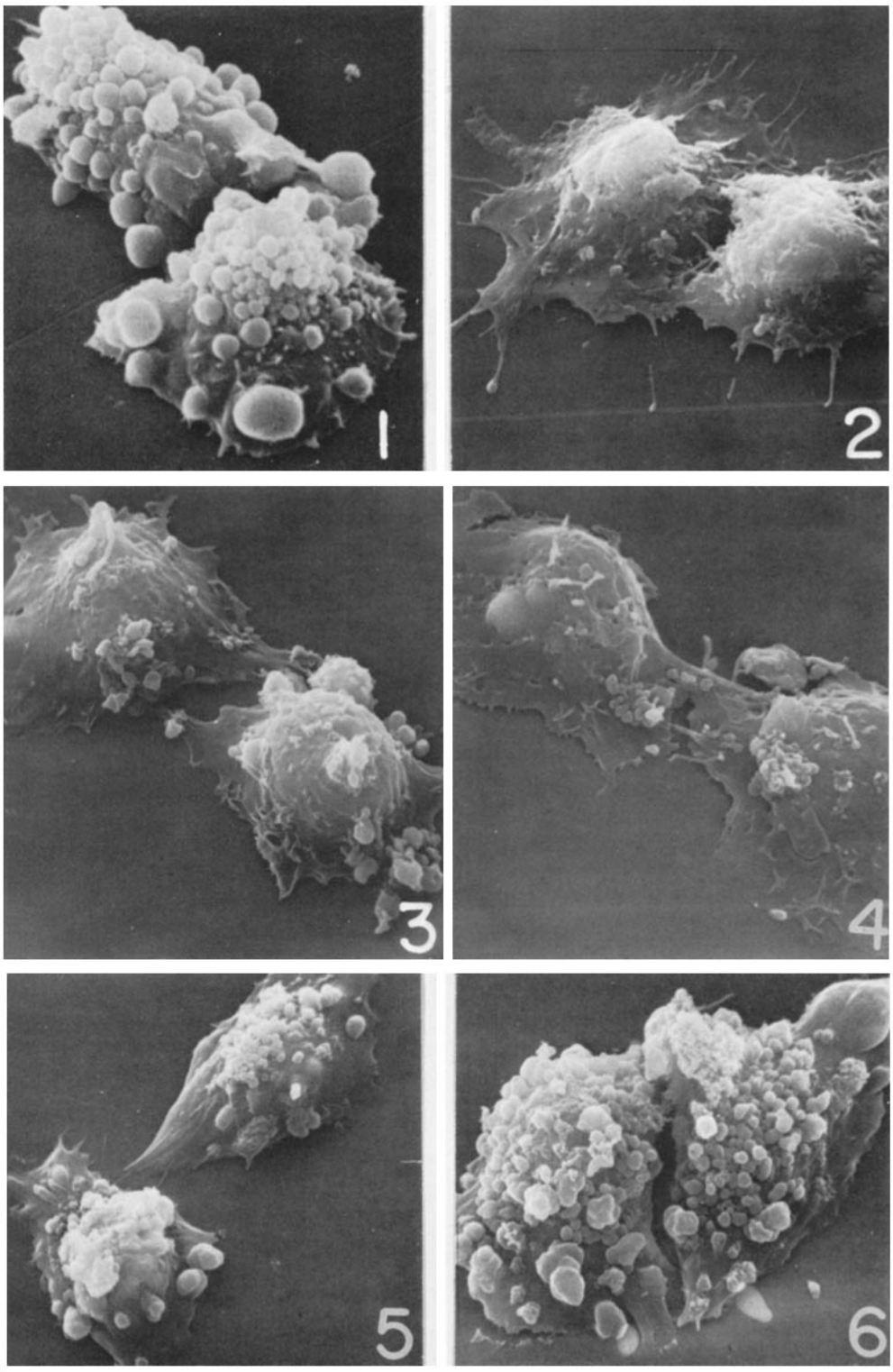

FIGURE 1 Typical G_1 cell. Note the rounded and highly blebbed morphology. × 1,000.
FIGURE 2 G_1 cells treated with CB for 5 min. × 1,000.
FIGURE 3 G_1 cells treated with CB for 20 min. × 1,000.
FIGURE 4 G_1 cells treated with CB for 1½ h. × 1,000.
FIGURE 5 G_1 cells treated with CB for 1 h and then released for 10 min by washing with conditioned media. × 1,000.
FIGURE 6 CB treated G_1 cells released for 10 min. × 1,000.

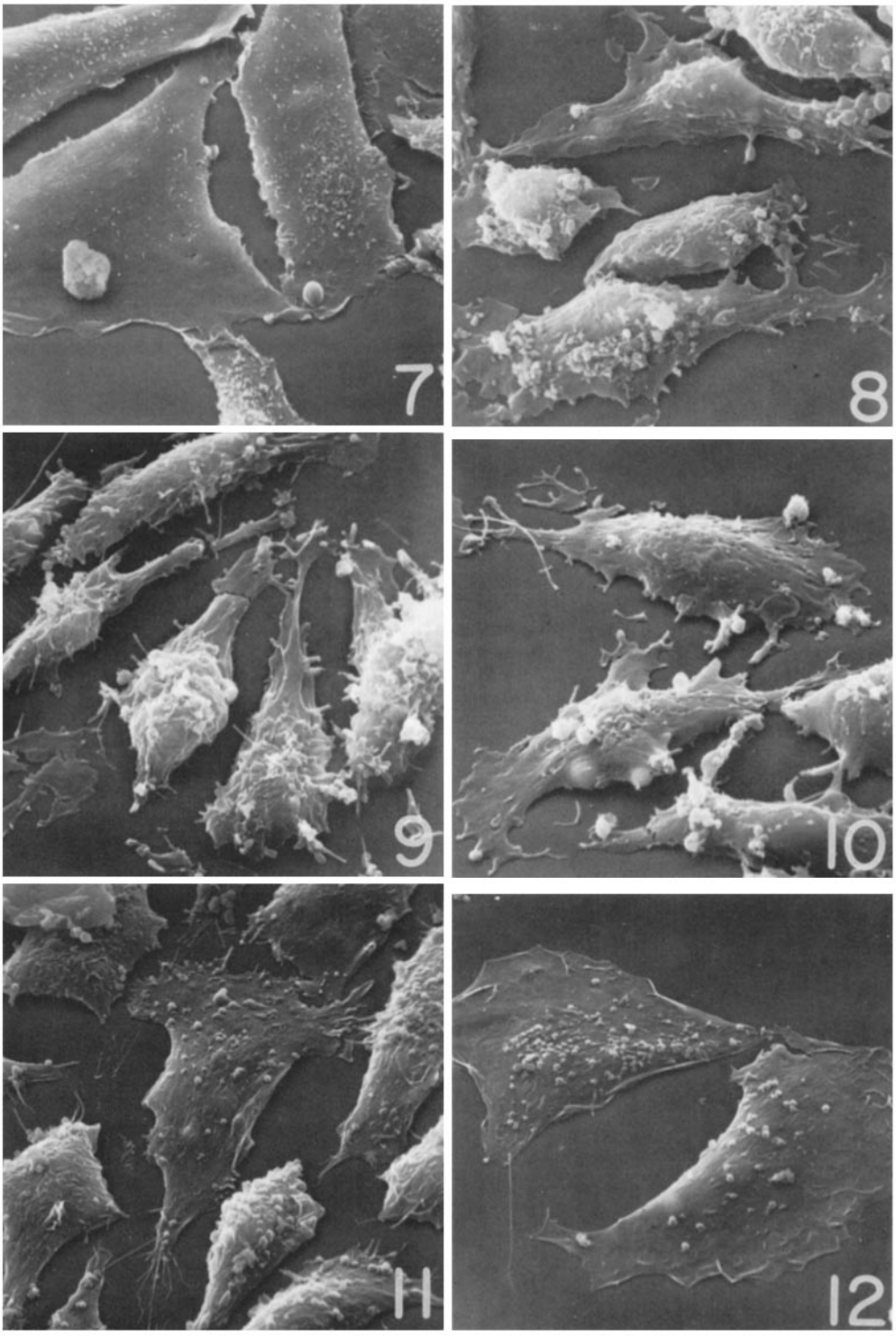

FIGURE 7 Untreated G_2 and S cells synchronized by isoleucine deprivation. × 1,000.

FIGURE 8 G_2 and S cells treated with CB for 10 min. × 1,000.

FIGURE 9 Same as Fig. 8 but treated for 20 min. × 1,000.

FIGURE 10 Same as Fig. 8 and 9 but treated for 15 min with 20 μg/ml. × 1,000.

FIGURE 11 S and G_2 cells treated with CB (5 μg/ml) for 1 h and released for 5 min. × 1,000.

FIGURE 12 Same as Fig. 11 but released for 20 min. × 1,000.

shape of the cell. Cells tend to round up, pull in their peripheral cytoplasm, and become stellate or arborized. These changes in shape have been observed by others at the light microscope level (Goldman, 1972; Sanger and Holtzer, 1972).

The initial response to CB is very rapid, being seen at the earliest time that cells were examined (2 min). Goldman (1972) also reports inhibition of ruffling upon addition of CB within minutes. Spooner et al. (1971) saw complete cessation of cell movement by 5 min. Zigmond and Hirsch (1972) found inhibition of 2-deoxyglucose uptake within 15 s of application of the drug. These rapid response times suggest that the mode of action of CB is a direct one not requiring secondary metabolic changes such as protein or RNA synthesis. We therefore propose that the rapid response of the cell surface reported here results from an interaction of CB with some component of the cell surface. This has been suggested on morphological grounds by Goldman (1972, 1973). In the preceding paper (Everhart and Rubin, 1973), we discussed biochemical evidence that CB interacts with the cell surface.

The speed of recovery of normal surface morphology after removal of CB suggests that the drug is rapidly washed away. This suggestion is consistent with the proposal that CB binds to the outside of the cell. However, other possibilities exist, such as that CB is rapidly broken down inside the cell. Cells recover the morphology that is characteristic of their stage in the cell cycle. This implies that, during treatment, information persists in the cytoplasm which can impart on the cell surface its characteristic morphology. This conclusion has also been reached by Goldman et al. (1973).

The slower response of overall cell shape due to peripheral cytoplasmic retraction could result from an effect of CB on microfilaments as proposed by Wessells et al. (1971). However, Goldman (1972) has shown that 5–10 μg/ml CB does not uniformly disrupt the microfilaments in baby hamster kidney (BHK-21) cells. Our results shed no light on this conflict. It is possible that binding of CB to the cell surface could affect the attachment of microfilaments without disrupting the microfilaments themselves.

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