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FEASIBILITY STUDY OF CYTOCHALASIN B ENUCLEATION OF CELLS

FINAL REPORT

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15 June 1973

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13. ABSTRACT

The goal of this study was to explore the feasibility of enucleating cells with Cytochalasi- B and subsequently to be able to transfer nuclei from one cell type to another. It was hoped that once such transfer had been made that hybrid cells composed of nuclei of one cell and cytoplasm of another could be useful in the study of a wide variety of problems which would involve the influence of cytoplasm on the nucleus and the converse. The results of this study and others indicate that enucleation can be accomplished with relative ease. In addition we found that a type of nucleus can be inserted into the cytoplasm of enucleated cells and survive. The problem of reinserting a heterologus (i.e. nucleus from another cell) nucleus into an enucleated cytoplasm has not been explored. Such reinsertion seems to remain possible.

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Summary

The goal of this study was to explore the feasibility of enucleating cells with Cytochalasin B and subsequently to be able to transfer nuclei from one cell type to another. It was hoped that once such transfer had been made that hybrid cells composed of nuclei of one cell and cytoplasm of another could be useful in the study of a wide variety of problems which would involve the influence of cytoplasm on the nucleus and the converse. The results of this study and others indicate that enucleation can be accomplished with relative ease. In addition we found that a type of nucleus can be inserted into the cytoplasm of enucleated cells and survive. The problem of reinserting a heterologus (i.e. nucleus from another cell) nucleus into an enucleated cytoplasm has not been explored. Such reinsertion seems to remain possible.

Body of Report

Introduction

S. B. Carter first described the biological effects of some cytochalasins, particularly Cytochalasin B. In his original report (1) he demonstrated that drug concentrations of 10 μg/ml would cause many mouse L cell nuclei to protrude on long slender cytoplasmic stalks. Such nuclei might, he suggested, be removed and could provide a chemical means of enucleation of such cells. If a means could be found to reunite different nuclei with different cells then a new technique could be available to study nucleo-cytoplasmic interaction in mammalian cells. A technique which had this potential was at this time being utilized by Dr. Henry Harris who was studying the problem of nucleo-cytoplasmic interaction. Using a phenomenon of cell fusion with Sendai virus discovered by Okada (2), Harris and his coworkers (3-8) had demonstrated that nuclei from red blood cells of the chicken (previously thought to be inert and incapable of function) when fused by means of this virus to human cancer cells (Hela cells) could become active, produce RNA which coded for and resulted in the production of chicken specific protein. He noted that the plasma membrane was necessary for cell fusion and that cell nuclei isolated by traditional fractionation procedures could not be utilized since they would not fuse to intact cells.

This information prompted us to repeat Carter's studies. We were able to induce enucleation in cultured L cells at doses of CB from 4 to 6 μg/ml. Since both techniques were reproducible several questions needed to be answered to make the use of both to accomplish nucleo-cytoplasmic interchange.

1) Did nuclei from CB treated cells retain plasma membrane?

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2) Could CB treated cells be fused?

3) Would enucleated cells be viable and would they support an inserted nucleus?

4) And finally could enucleated cells and nuclei produced by cytochalasin treatment be fused and produce a viable hybrid?

The answer to the first question came through use of the electron microscope. Although light microscopy suggested that plasma membrane existed and that these nuclei excluded Trypan blue, final proof was the ultrastructural conformation of the presence of plasma membrane around the nucleus and virtually complete exclusion of cytoplasmic organelles from the small amount of cytoplasm surrounding the extruded nucleus. Since cytoplasmic contamination was a possible problem in nuclear exchange experiments it was clear that if such nuclei could be used that most of the cytoplasm had been eliminated by nuclear extrusion. The second question was answered easily by comparing fusion efficiency of virus with CB treated and untreated cells. Removal of CB from the media with subsequent fusion resulted in the same efficiency of fusion in treated and untreated cells. The fact that CB action on numerous processes has subsequently been shown to be rapidly reversible is additional evidence that this result is correct (9).

To answer the third question we needed a system that would provide clear evidence that enucleated cells could support the metabolism of the nucleus to be introduced. Harris and coworkers had used chicken erythrocyte nuclei in his Sendai cell virus fusion system. These experiments provided the model we were looking for since 1) the chicken erythrocyte nucleus provided a distintive morphologic marker, 2) the nucleus was metabolically inactive in the parent cell in that it failed to incorporate precursors of DNA or RNA into macromolecules, and 3) the events of activation were well documented and the following sequential changes could be observed: a) nuclear swelling, b) incorporation of tritiated thymidine and uridine into the nucleus, c) appearance of nucleoli, d) export of uridine label to the cytoplasm and e) appearance of chicken specific protein in the hybrid cell. Technical details of experimental methods and their results are described in reference 14.

This data indicated that chicken erythrocyte nuclei when fused to enucleate cells appeared in the cytoplasm of these cells, underwent swelling, nucleoli appeared and uridine label appeared in the cytoplasm (10). These findings indicated that the technique of enucleation and fusion with a heterologus nucleus is feasible. Subsequently others have shown similar results (11).

In addition to these authors other workers (12,13) including our own unpublished experiments have shown that centrifugation will increase the number of enucleated cells. However, no one has been able to this point, to exchange cytochalasin enucleated nuclei. In addition to completion of this contract, our work on understanding the mode of CB action has been aided immeasurably by availability of equipment purchased by this contract (see reference 9).

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