THE REQUIREMENT FOR BIOTIN IN MOUSE FIBROBLAST L-CELLS CULTURED ON SERUMLESS MEDIUM

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SUMMARY: Mouse L-cells, Clone 929, were adapted to growth on a chemically defined medium, Waymouth's Medium MAP 954/1, in the absence of serum. When biotin was excluded in the preparation of this medium, the resulting medium proved to be incapable of sustaining continuous cell proliferation: A biotin requirement was therefore demonstrable in these cells within a three week period.

The nutritional requirement for biotin has been well established in the whole animal (1), and a number of studies have been made on the pathological symptoms (2) as well as the changes in overall metabolism (3-7) that result from its prolonged exclusion from the animal diet. That biotin should also constitute an essential nutrient for the continued growth of individual cells in culture would be predicted from its obligatory involvement in carbohydrate and lipid metabolism as the prosthetic group of certain key enzymes (8-10). However, contrary to such expectations, the direct demonstration of a biotin requirement in a cell culture system has not yet been reported (11).

Since most cultured cells must be grown on a medium containing either serum or some other uncharacterized substance of biological origin, the elimination of the last traces of biotin from such an experimental system is, at best, difficult (12). The results presented in this paper were obtained using a line of cells that had previously been adapted to grow on a chemically defined medium containing no uncharacterized biologically derived fraction. With such

an experimental system a biotin requirement was demonstrable in these cells within a three week period.

Materials and Methods: The cells used for this experiment were mouse fibroblast L-cells, and the original stock of cells was the gift of Mr. Robert C. Veomett of the Department of Radiology at the UCLA Medical Center. Since the cells at that time were routinely being cultured on Eagle's Minimum Essential Medium supplemented with 20% fetal bovine serum, they first had to be conditioned to grow on a chemically defined medium in the absence of serum. The medium finally selected for the growth of the cells under these conditions was Waymouth's Medium MAP 954/1 (13), which was made up fresh in the laboratory before use. The cells were cultured in 250 ml. Falcon plastic tissue culture bottles, and 20 ml. of medium was used per bottle. Incubation was at 37 C in an atmosphere of 95% air and 5% CO_2. Subculturing was accomplished, after a preliminary washing with 20 ml. of Harary's Saline A (14), by a 5 minute incubation at 37 C in 3-5 ml. of 0.01% trypsin solution in Saline A. Upon disattachment, the cells were dispersed with a pipette and seeded directly into 20 ml. of medium. Since the cells were always found to be plated within 30 minutes after inoculation, a change of fresh medium was given at that time. Cell counts were routinely made on a small aliquot of the trypsinized cell suspension using a hemocytometer.

Results and Discussion: The experiment was carried out in the following manner. 1.1×10^6 were plated into each of 2 bottles, one containing the regular growth medium and the other containing medium deficient in biotin, both media having been made up and sterilized on the same day. The respective media were changed after the first 30 minutes and thence every 2-3 days. After a period of 8 days, a visible difference in cell density was observable between the two groups since only the control group of cells was found to be approaching a state of confluence. On the seventeenth day, each of the 2 bottles was subcultured to 3 bottles, and the 6 resulting bottles were allowed to grow for another 4 days. The cells were then harvested from all 6 bottles with trypsin, and cell counts were determined.

Cell counts of 1.4, 1.5, and 2.3 x 10^6 cells / bottle were recorded for the 3 bottles of the control group, whereas fewer than 5 cells / bottle could be found in all 3 bottles of the biotin-deficient group. The experiment was then repeated starting out initially with 2 bottles of cells per experimental group, and comparable results were obtained after the second subculturing.

Since throughout each of these experiments all general procedures as well as specific handling techniques were performed identically for both of the experimental groups, the observed cessation of growth in the biotin-deficient group of cells can only be attributed to the lack of biotin in the culture medium. The authors, therefore, conclude that mouse L-cells cultured on serum-less medium exhibit an express biotin requirement for their continued proliferation in culture. That the need for biotin as an essential nutrient should have rapidly become manifest in cells cultured under these conditions is indeed reasonable since endogenous lipogenesis constitutes their only source of fatty acids for the synthesis of new cell membranes and biotin is accordingly an obligatory cofactor for the acetylSCoA carboxylase reaction (8), the enzymatic step initiating the pathway of saturated and monounsaturated fatty acid synthesis in the animal.

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