ISOLATION OF A MAMMALIAN CELL MUTANT RESISTANT TO 25-HYDROXY CHOLESTEROL

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A mutant of the Chinese Hamster Ovary cell line, CHO-K1, has been isolated which is resistant to killing by 25-hydroxy cholesterol in the absence of cholesterol. There is no effect on acetate incorporation into cholesterol by 25-hydroxy cholesterol in the mutant under conditions in which incorporation is inhibited in the parent cell. The mutant also appears to be defective in the regulation of cholesterol synthesis by serum cholesterol.

INTRODUCTION

Certain oxygenated derivatives of cholesterol have been shown to be potent inhibitors of sterol synthesis in cultured mammalian cells (1). These compounds act by specifically depressing the level of HMG-CoA reductase activity in these cells by an as yet unidentified mechanism which does not operate on the enzyme molecule itself.

Because of this specific effect, prolonged incubation of fibroblasts with these compounds results in cessation of growth and death unless an appropriate sterol is added to the growth medium (2). In this report, we describe a mutant of the Chinese hamster ovary cell line (CHO-K1) which is resistant to the killing effects of 25-hydroxy cholesterol in the absence of cholesterol.

METHODS AND MATERIALS

1-C^{14} acetate (specific activity 58m Ci/m mol) was obtained from Amersham-Searle. 5-^{3}H mevalonate (dibenzyl ethylene diamine salt; specific activity 5 Ci/m mol) was obtained from New England Nuclear. Cholest-5-ene-3β, 25-diol (25-hydroxy cholesterol) was obtained from Steraloids, (1α, 2α(n)-^{3}H) - cholesterol (specific activity 43 Ci/m mol) was obtained from Amersham-Searle.

Cells and Medium

The CHO-K1 cell used in these experiments is that described by Kao and

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Puck (3,4). This cell has proved to be quite amenable to the isolation of mutants (5). The cells were grown on Ham's F12 (6) supplemented with 8% fetal calf serum (F12FC8) or with 4% cholesterol-poor fetal calf serum. Cholesterol-poor serum was prepared by removing the lipoproteins by flotation at 200,000 xg for 48 hours at a density adjusted to 1.215 with KBr. This serum was dialyzed exhaustively prior to use.

Mutagenesis and Mutant Isolation

After mutagenesis with 400 µg/ml of ethylmethane sulfonate for 16 hours, 10^6 cells were plated at 25,000 cells per plate on 60 mm plates in F12FC8. After overnight incubation of these plates, the medium was changed to F12 supplemented with 500 µg/ml delipidated serum protein (7) and 0.5 µg/ml 25-hydroxy cholesterol. The plates were then left to incubate undisturbed for 10 days resulting in the appearance of several clones - most of which contained 20 to 30 cells but one which had several hundred cells. It is this clone which is the subject of this report.

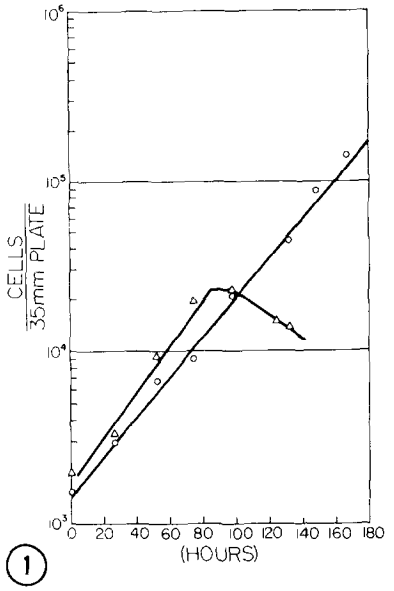
Lipid Analysis

Cellular lipids were extracted by the method of Bligh and Dyer (8). Isolation of cholesterol and cholesterol ester from these extracts was by the method of Freeman and West (9) on commercial silica gel thin layer chromatographic plates (Brinkman). Saponification was performed at 55°C in 0.2 ml of 5% ethanolic KOH. Separation of fatty acid and cholesterol after saponification was either by extraction from hexane with 5% $NaHCO_3$ or by silica gel thin layer chromatography in 80:20:1, petroleum ether/diethyl ether/acetic acid. Lipids were visualized on chromatography plates either by radio-thin-layer chromatographic scanner (Packard) or by use of iodine vapor in the presence of mass carrier.

RESULTS AND DISCUSSION

The growth of the putative resistant mutant (CR1) and the parent cell of cholesterol-poor medium supplemented with 25-hydroxy cholesterol is shown in Figure 1 and demonstrates the resistant phenotype. The karyotype of CR1 is identical to that of its parent cell type (CHO-K1).

It would be expected that the resistance to the killing effects of 25-hydroxy cholesterol would be reflected by a resistance to the effects of this compound on the conversion of labelled acetate to cholesterol. In Figure 2, the kinetics of acetate conversion to cholesterol in the mutant and parental cell are presented. Under the conditions shown in Figure 2, in which cells are incubated in the absence of serum or other exogenous source of cholesterol, cholesterol synthesis should be maximal. Clearly 25-hydroxy cholesterol has virtually no effect on the mutant whereas it greatly reduces cholesterol synthesis in the parent cell. Furthermore, the rate of cholesterol synthesis



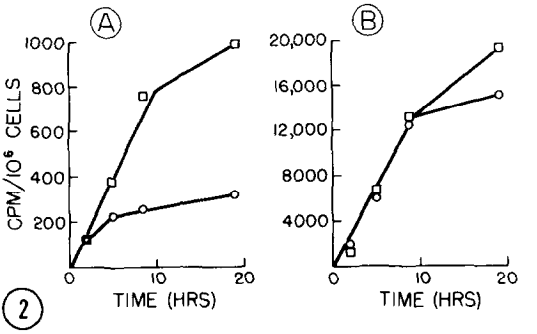


Figure 1: Growth of a CHO-K1 (Δ) and CR1 (O) on cholesterol-poor medium supplemented with 0.1 μg/ml of 25-hydroxy cholesterol. Cells were innoculated into 35 mm plates at an initial titer of 2,000 cells/plate, 60 hours prior to the first measurement. The cells per plate were determined by removing the cells from the plate by trypsinization, and counting them with a Coulter Counter.

Figure 2: Effect of 25-hydroxy cholesterol on the conversion of 1-^{14}C acetate to cholesterol in CHO-K1 (panel A) and CR1 (panel B). Cells were grown to a titer of approximately 5x10^6 cells in 100mm plates in F12FC8. The medium was then changed to F12 (□) or F12 supplemented with 0.5 μg/ml 25-hydroxyl cholesterol (O). After 4 hours incubation, 10 μCi of 1-^{14}C acetate was added to each plate and the cells harvested, counted and their lipids extracted at the times shown. The lipids were saponified and cholesterol and fatty acids isolated by thin-layer chromatography and counted. Each point is the average of two determinations. CHO-K1 and CR1 had similar patterns of incorporation into fatty acid which were unaffected by the addition of 25-hydroxy cholesterol.

in CR1 is 20-fold greater than even the fully de-repressed level seen in CHO-K1. We have no explanation at this time for this observation, but are currently testing several hypotheses.

The ability of cholesterol to regulate cholesterol synthesis in the mutant was also subjected to preliminary examination. We find that when CR1 and CHO-K1 are grown in cholesterol containing medium (F12FC8) in the presence of various labelled cholesterol precursors a defect in the regulation of cholesterol synthesis at the level of HMG-CoA reductase is indicated. These exper-

TABLE 1

Relative Incorporation of Cholesterol Precursors into Cholesterol and the Cholesterol Moiety of Cholesterol Ester in CHO-K1 and CR1

Precursor	Product	Relative Incorporation dpm-mg protein^{-1}:CR1/CHO-K1
acetate	cholesterol	13
acetate	cholesterol ester	4
mevalonate	total cholesterol	1
exogenous cholesterol	cholesterol	1
exogenous cholesterol	cholesterol ester	1

Cells were grown for four generations in the presence of 50 uci of labelled precursor (1-^{14}C-acetate, 5-^{3}H mevalonate, or [1α, 2α(n)-^{3}H] cholesterol).

iments (Table 1) show a greatly increased incorporation of acetate into cholesterol in CR1 over CHO-K1. In contrast, incorporation of mevalonate into cholesterol or of exogenous cholesterol into cellular cholesterol is the same in the two cells. Thus, although cholesterol uptake in CR1 is apparently normal, acetate incorporation is not comparably regulated.

The regulation of HMG-CoA reductase levels by cholesterol is an area of great interest for fundamental cell biology and for understanding of the diseases in which cholesterol is involved, and most current work does point to the level of intracellular cholesterol as being of critical regulatory significance (10,11). The molecular mechanism by which cholesterol affects HMG-CoA reductase activity, however, remains obscure. It is hoped that further analysis of CR1 and other somatic cell mutants in cholesterol synthesis isolated in these laboratories will play an important role in elucidating these mechanisms.

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