Activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase does not respond to ubiquinone uptake in cultured cells

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The cellular content of ubiquinone was increased approx. 10-fold by incubation of neuroblastoma cells in medium containing exogenous ubiquinone. Under these conditions the activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, assayed after preincubation of cell homogenates with or without fluoride, was not suppressed. Similar results were obtained with human skin fibroblast cultures to which free ubiquinone or low-density lipoprotein–ubiquinone complex had been added. Consistent with the lack of suppression of HMG-CoA reductase, the rate of incorporation of [1-¹⁴C] acetate into ubiquinone was not diminished in cells exposed to exogenous ubiquinone. Measurements of [³H]mevalonolactone incorporation into cellular ubiquinones indicated that exogenous ubiquinone did not affect ubiquinone synthesis at a point in the pathway distal to the formation of mevalonate. The results suggest that cultured mammalian cells lack an end-product 'feedback' mechanism for regulation of HMG-CoA reductase in response to ubiquinone uptake.

INTRODUCTION

Mevalonate occupies a central position as a precursor in the branched pathway for isoprenoid biosynthesis in eukaryotic cells (Brown & Goldstein, 1980). End-products of this pathway, derived wholly or in part from mevalonate, include cholesterol (Schroepfer, 1981), dolichol (Gough & Hemming, 1970; Martin & Thorne, 1974), the polyisoprene side chain of ubiquinone (Rudney, 1977), isopentenyladenosine residues of tRNA (Hall, 1970; Faust *et al.*, 1980), the farnesyl moiety of haem *a* (Weinstein *et al.*, 1986), and heretofore uncharacterized isoprenoid groups associated with specific cellular polypeptides (Schmidt *et al.*, 1984; Sinensky & Logel, 1985; Maltese & Sheridan, 1986).

Mevalonate is formed in the reaction catalysed by HMG-CoA reductase, an enzyme that plays a central role in the regulation of cholesterol biosynthesis (Rodwell *et al.*, 1976; Schroepfer, 1981). Under certain conditions, changes in the activity of HMG-CoA reductase also affect the rates of synthesis of dolichol (Mills & Adamany, 1978; James & Kandutsch, 1979, 1980) and ubiquinone (Faust *et al.*, 1979; Nambudiri *et al.*, 1980; Ranganathan *et al.*, 1981). The 'feedback' regulation of HMG-CoA reductase by LDL cholesterol (Goldstein & Brown, 1977; Chin *et al.*, 1982; Liscum *et al.*, 1983) and oxygenated precursors or derivatives of cholesterol (Kandutsch *et al.*, 1978; Gibbons *et al.*, 1980; Taylor *et al.*, 1984) is well established. Free cholesterol also has been reported to suppress the activity of HMG-CoA reductase (Brown & Goldstein, 1974; Brown *et al.*, 1974; Volpe & Hennessy, 1977), although there remains some question as to whether this effect is mediated by cholesterol itself or by trace amounts of oxysterol contaminants (Kandutsch & Chen, 1973; Chen

et al., 1975; Breslow *et al.*, 1975). Most recently, certain unidentified non-sterol derivatives of mevalonate have been implicated in the regulation of HMG-CoA reductase (Peffley & Sinensky, 1985; Watson *et al.*, 1985).

Ubiquinone (coenzyme Q) functions as a mobile carrier of reducing equivalents in the mitochondrial electron-transport chain (Crane, 1977; Trumpower, 1981; Lenaz *et al.*, 1985). Significant quantities of ubiquinone are present in the circulation (Ogasahara *et al.*, 1985; Folkers *et al.*, 1985), and it has been shown that administration of ubiquinone in the diet can raise the concentration of ubiquinone in the liver (Krishnaiah & Ramasarma, 1970; Ranganathan & Ramasarma, 1975) and cardiac muscle (Littarru *et al.*, 1970). Nevertheless, studies conducted *in vivo* have not provided conclusive evidence as to whether the uptake of extracellular ubiquinone regulates the endogenous synthesis of this isoprenoid via an end-product 'feedback' effect on HMG-CoA reductase, analogous to that described for sterols. In the present study cultured cells were used to address this issue.

MATERIALS AND METHODS

Materials

C1300 murine neuroblastoma cells (Neuro 2A) were obtained from the American Type Culture Collection, Rockville, MD, U.S.A. Human fibroblasts were derived from a skin biopsy from a normal volunteer. Tissue-culture medium and serum were purchased from Gibco, Grand Island, NY, U.S.A. [2-³H]Mevalonolactone (1.28 Ci/mmol) and [1-¹⁴C]acetate (57 mCi/mmol) were obtained from Amersham Corp., Arlington Heights, IL, U.S.A. Cholesterol was obtained from Steraloids Inc.,

Abbreviations used: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; LDL, low-density lipoprotein; LPPS, lipoprotein-poor fetal-calf serum; PBS, phosphate-buffered saline (Dulbecco & Vogt, 1954).

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Wilton, NH, U.S.A., and was used without further purification. Ubiquinones, human serum LDL and other reagents were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Cell culture

Neuroblastoma cells and skin fibroblasts were maintained in monolayer culture in Dulbecco's modified Eagle's medium, supplemented with 10% (v/v) fetal-calf serum as described previously (Maltese & Sheridan, 1985). Lipoprotein-poor fetal-calf serum (LPPS) was prepared by removing the LDL fraction by centrifugal flotation as described by Havel *et al.* (1955). The LPPS was dialysed extensively against Dulbecco's phosphate-buffered saline (PBS) and sterilized by passage through a 0.22 μ m filter before it was added to the culture medium.

Ubiquinone preparations

Commercial preparations of ubiquinone-10 or ubiquinone-9 were purified routinely by two-dimensional t.l.c. (Maltese & Aprille, 1985) to remove any contaminating sterols or squalene derivatives. The purified ubiquinones were dissolved in ethanol and stored in the dark at -80°C under N_2 .

LDL was reconstituted with ubiquinone by the procedure of Krieger *et al.* (1979). The final LDL-ubiquinone complex (after Millipore filtration) contained 603.4 nmol of ubiquinone-10/mg of protein (mass ratio = 0.519), compared with the native LDL, which contained only 8.7 nmol of ubiquinone/mg of protein. Cholesterol was undetectable in the reconstituted LDL-ubiquinone complex when assayed with a commercial colorimetric kit (Stanbio Laboratory, San Antonio, TX, U.S.A.). The native LDL had a cholesterol/protein mass ratio of 0.340.

[^3H]Ubiquinone for use in studies of ubiquinone binding to serum proteins was obtained by incubating eight 75 cm^2 neuroblastoma cultures for 24 h with [^3H]mevalonolactone (5 $\mu\text{Ci}/\text{ml}$ of medium). Cells were harvested and, after addition of 30 μg of unlabelled ubiquinone-10 as a carrier, the radioactive ubiquinone generated by the cells was extracted, purified by t.l.c., and quantified spectrophotometrically as described previously (Maltese & Aprille, 1985). The final specific radioactivity of the [^3H]ubiquinone eluted from the silica gel was 104 mCi/mmol.

Binding of ubiquinone to serum protein

To determine the relative amount of free ubiquinone that became associated with serum proteins in the tissue culture medium, a 2.5 ml sample of medium (containing [^3H]ubiquinone at a final concentration of 11.6 μM) was mixed with an equal volume of 50% (w/v) trichloroacetic acid, and the resulting precipitate was removed by centrifugation at 10000 g for 20 min. The precipitate was washed twice with 5 ml of 10% trichloroacetic acid and dissolved in 2 ml of 0.5 M-KOH. A 0.5 ml portion of the KOH solution was mixed with 0.6 ml of 0.5 M-HCl and 10 ml of 3a70B scintillation fluid (Research Products International, Elk Grove, IL, U.S.A.), and the samples were counted for radioactivity in a Packard Tri-Carb liquid-scintillation spectrometer. The trichloroacetic acid-precipitable radioactivity was expressed as a fraction of the total radioactivity originally added to the culture medium in the form of free ubiquinone. When equal

amounts of medium containing [^3H]ubiquinone were centrifuged without prior addition of trichloroacetic acid, there was no radioactivity in the slight residue at the bottom of the tube. Moreover, when [^3H]ubiquinone was added to samples of culture medium that did not contain serum, no radioactivity was precipitated by addition of trichloroacetic acid.

Cellular uptake of ubiquinone

After incubation of cells in medium containing exogenous ubiquinone, the uptake of ubiquinone was determined either in whole cells that had been washed four times with PBS or in mitochondria-enriched subcellular fractions prepared by differential centrifugation (Maltese & Aprille, 1985). Extraction of ubiquinone and quantification of ubiquinone by dual-wavelength spectroscopy of oxidized versus reduced samples were performed as described previously (Maltese & Aprille, 1985). Protein was quantified by the method of Lowry *et al.* (1951), and the mitochondrial marker enzyme, succinate dehydrogenase, was assayed by a spectrophotometric method (Singer, 1974; Maltese & Aprille, 1985).

HMG-CoA reductase assay

Total cellular enzyme activity was determined by measuring the NADPH-dependent conversion of [^3H]HMG-CoA into [^3H]mevalonate as described previously (Maltese *et al.*, 1980). To determine the relative amount of 'latent' enzyme activity (i.e. enzyme that can be activated by preincubation in the absence of a phosphatase inhibitor), cells were homogenized and assayed with or without the addition of 50 mM-NaF to all solutions (Maltese *et al.*, 1980). One unit of enzyme activity equalled 1 pmol of mevalonate formed/min at 37°C .

Ubiquinone synthesis

The rate of synthesis of ubiquinone in cultured neuroblastoma cells or fibroblasts was determined by measuring the incorporation of radioactivity into ubiquinone in cultures that were incubated for 4 h with [$2\text{-}^3\text{H}$]mevalonolactone or [$1\text{-}^{14}\text{C}$]acetate (see Figure legends for concentrations). Ubiquinones were isolated from the cell extracts by two-dimensional t.l.c. on silica-gel plates (Maltese & Aprille, 1985), and the radioactivity in the ubiquinone zone scraped from each chromatogram was determined by counting in a liquid-scintillation spectrometer. The results were expressed on the basis of the amount of cellular protein present in the cell homogenate extracted for ubiquinone.

RESULTS

Cellular uptake of ubiquinone

We have shown that inhibition of HMG-CoA reductase in C1300 murine neuroblastoma cells results in a marked decline in the mitochondrial ubiquinone concentration (Maltese & Aprille, 1985), indicating that synthesis *de novo* represents an important source of ubiquinone in this cell line. The studies by Volpe & Hennessy (1977) demonstrated that, unlike some transformed tissues (Siperstein & Fagan, 1964; Sabine *et al.*, 1967), murine neuroblastoma cells retain the capacity for 'feedback' regulation of HMG-CoA reductase by exogenous free sterols and LDL. Thus the C1300 (Neuro

Fig. 1. Uptake of exogenous ubiquinone by cultured neuroblastoma cells

Cells were seeded in 175 cm² flasks at an initial density of 20000 cells/cm². After 48 h the medium was changed and ubiquinone-10 was added to yield a final concentration of 11.6 μ M. Duplicate cultures were harvested at each of the indicated time points, and quantification of ubiquinone was performed on whole cells as described in the Materials and methods section.

Table 1. Effect of increasing extracellular concentration of ubiquinone on the ubiquinone content of mitochondria in neuroblastoma cells

Cells were seeded in 175 cm² culture flasks at an initial density of 20000 cells/cm². After 2 days the medium was changed and ubiquinone-10 was added to yield the final concentrations listed below. The cells were incubated with ubiquinone for 18 h before being harvested for subcellular fractionation and quantification of ubiquinone in the mitochondria-enriched fraction (see the Materials and methods section). To correct for variations in the recovery and purity of mitochondria, ubiquinone content was expressed on the basis of succinate dehydrogenase (SDH) activity (1 unit of activity equals 1 nmol of 2,6-dichloroindophenol oxidized/min at 30 °C) as well as on the basis of protein. The fold enrichment of SDH specific activity in the mitochondrial fractions was 4.2, relative to the crude cell homogenates.

Concn. ubiquinone added to the medium	Concn. of ubiquinone in the mitochondrial fraction	
	(nmol/mg of protein)	(nmol/unit of SDH)
0	0.98	0.015
0.6 μ M	1.56	0.025
1.7 μ M	3.69	0.053
11.6 μ M	12.78	0.187

2A) cells appeared to be a suitable system in which to investigate the possibility that the activity of HMG-CoA reductase responds to the concentration of exogenous ubiquinone.

When neuroblastoma cells were incubated in medium

Fig. 2. Proportion of added ubiquinone associated with serum proteins in the culture medium

Tubes containing 5 ml portions of Dulbecco's medium with either 10% (v/v) whole fetal-calf serum (■) or 10% (v/v) LPPS (□) were warmed to 37 °C. [³H]Ubiquinone (104 mCi/mmol) was added to each portion of medium to give a final concentration of 11.6 μ M, and the samples were incubated for 24 h. At the designated intervals after addition of ubiquinone, the percentage of the total radioactivity that could be precipitated with trichloroacetic acid was determined as described in the Materials and methods section.

containing 11.6 μ M-ubiquinone, they accumulated ubiquinone in a time-dependent manner, so that by 24 h the cellular content of ubiquinone, expressed on the basis of total protein, increased more than 10-fold (Fig. 1). Although the cells were washed several times before lipid extraction, we considered the possibility that some of the increase in ubiquinone content was due to non-specific lipophilic adsorption of the exogenous ubiquinone to the cell membrane. However, the fact that the final ubiquinone concentration in the washed cell pellet was approx. 200 μ M (compared with 11.6 μ M in the culture medium) suggested that most of the increase in cellular ubiquinone resulted from active uptake and sequestration of the extracellular isoprenoid. The data in Table 1 confirm that the ubiquinone content of the mitochondria-enriched subcellular fraction increased substantially in conjunction with the addition of progressively greater amounts of ubiquinone to the culture medium.

Ubiquinone was added to the culture medium in the free form. However, the data depicted in Fig. 2 indicate that most of the ubiquinone quickly became associated with serum proteins, and remained so throughout the incubation period. Since there was no significant difference in the proportion of protein-bound ubiquinone in the medium containing whole serum or LPPS (Fig. 2), the ubiquinone apparently was associated with proteins other than LDL under these conditions.

Effects of exogenous ubiquinone on HMG-CoA reductase activity

To assess the effect of increased uptake of ubiquinone on the activity of HMG-CoA reductase, neuroblastoma cells were incubated for 24 h with ubiquinone-9, the

Table 2. Effect of ubiquinone on the activity of HMG-CoA reductase in cultured neuroblastoma cells and skin fibroblasts

Neuroblastoma cells were seeded in 75 cm² flasks at 30 000 cells/cm². Fibroblasts were seeded in 25 cm² flasks at 100 000 cells/cm². After 24 h, cultures were fed with fresh medium containing either 10% (v/v) whole fetal-calf serum or 10% (v/v) lipoprotein-poor fetal calf serum (LPPS). Then 24 h later (for neuroblastoma cells) or 48 h later (for fibroblasts) the indicated additions were made to the culture medium. After 24 h, all cultures were harvested and assayed for HMG-CoA reductase activity (see the Materials and methods section). All values are means ($\pm$ S.E.M.) of separate determinations performed on three cultures.

Culture conditions		HMG-CoA reductase activity (units/mg of protein)		
		– NaF	+ NaF	Latent activity (%)
Neuroblastoma cells	Whole serum	36.6 $\pm$ 0.8	–	–
	Whole serum + ubiquinone-9 (11.6 μ M)	37.7 $\pm$ 3.7	–	–
	LPPS	142.2 $\pm$ 5.2	48.6 $\pm$ 3.3	66
	LPPS + ubiquinone-9 (11.6 μ M)	158.2 $\pm$ 3.9	57.4 $\pm$ 12.0	64
Skin fibroblasts	Whole serum	4.8 $\pm$ 0.2	–	–
	Whole serum + ubiquinone-10 (11.6 μ M)	8.2 $\pm$ 1.2	–	–
	LPPS	56.6 $\pm$ 5.9	18.1 $\pm$ 0.7	68
		(29.9 $\pm$ 4.0)*	–	–
	LPPS + ubiquinone-10 (11.6 μ M)	44.3 $\pm$ 3.5	19.6 $\pm$ 1.5	56
		(39.6 $\pm$ 5.2)*	–	–
	LPPS + LDL–ubiquinone (9.8 μ M–ubiquinone)	85.1 $\pm$ 4.0	33.9 $\pm$ 2.3	60
	LPPS + LDL (65 μ g of cholesterol/ml of medium)	8.5 $\pm$ 0.4	2.8 $\pm$ 0.9	67
	LPPS + cholesterol (65 μ g/ml of medium)	6.0 $\pm$ 2.6	3.8 $\pm$ 0.7	37

* Values derived from a separate experiment with the same cell line.

predominant end-product of the ubiquinone pathway in murine tissues. Under culture conditions that promote maximum expression of HMG-CoA reductase activity (i.e. 10% LPPS substituted for whole fetal-calf serum), the addition of exogenous ubiquinone did not suppress the activity of the reductase to an extent that would be consistent with a 'feedback' regulatory mechanism (Table 2). Similar results were obtained with cultures that were grown with whole fetal-calf serum (Table 2).

Whole-cell homogenates from a variety of tissues and cultured cell lines appear to contain substantial amounts of HMG-CoA reductase in a 'latent' phosphorylated form, which can be activated by preincubation of the homogenates in the absence of phosphatase inhibitors (Berndt *et al.*, 1976; Nordstrom *et al.*, 1977; Saucier & Kandutsch, 1979; Brown *et al.*, 1979). If the intracellular accumulation of ubiquinone triggers an increase in the proportion of enzyme in the phosphorylated (physiologically inactive) form, this could not be detected by assaying the fully activated HMG-CoA reductase in preincubated cell extracts. To test this possibility, neuroblastoma cells were homogenized, preincubated and assayed for HMG-CoA reductase in the presence of the phosphatase inhibitor, NaF. Approx. 60–65% of the HMG-CoA reductase activity in neuroblastoma cells was in the latent form (Table 2). When ubiquinone was added to the medium, there was no change in the proportion of latent activity relative to total activity (Table 2), suggesting that ubiquinone does not cause an alteration of the catalytic state of the reductase via a phosphorylation mechanism.

Similar studies with normal human skin fibroblasts indicate that the absence of 'feedback' suppression of HMG-CoA reductase in response to increased extracellular ubiquinone concentration is not a unique feature of the neuroblastoma cell line. Consistent with previous reports (Brown *et al.*, 1974; Brown & Goldstein, 1974), the activity of the reductase in fibroblasts was greatly decreased by addition of either free cholesterol or LDL

Table 3. Ubiquinone concentration in fibroblasts incubated with medium containing ubiquinone or LDL–ubiquinone

Fibroblasts were seeded in four 75 cm² cultures at an initial density of 100 000 cells/cm². After 4 days of growth in medium supplemented with 10% (v/v) LPPS, the medium was replaced with medium derived from the fibroblast cultures assayed for HMG-CoA reductase (see Table 2). Each 75 cm² culture received medium pooled from three 25 cm² cultures. After 24 h incubation with medium containing the indicated supplements, the cultures were harvested, washed and assayed for ubiquinone (see the Materials and methods section).

Culture conditions	Cellular ubiquinone concn. (nmol/mg of protein)
LPPS	0.20
LPPS + LDL	0.16
LPPS + LDL–ubiquinone	2.66
LPPS + ubiquinone	3.00

Fig. 3. Incorporation of labelled precursors into ubiquinone in cultured neuroblastoma cells and skin fibroblasts

Neuroblastoma cells were seeded in 75 cm² flasks at a density of 30 000 cells/cm² and grown for 48 h. Fibroblasts were seeded in 75 cm² flasks at 25 000 cells/cm² and grown for 72 h. Cells were incubated with either [2-³H]mevalonolactone (a) or [1-¹⁴C]acetate (b) for the indicated periods of time, and the incorporation of radioactivity into cellular ubiquinone was determined as described in the Materials and methods section. For the neuroblastoma cultures (○) the concentration of [1-¹⁴C]acetate in the medium was 2 μCi/ml, and the concentration of [2-³H]mevalonolactone was 2 μCi/ml. For fibroblast cultures (●) the concentration of [2-³H]mevalonolactone was increased to 4 μCi/ml of medium. Each point represents the mean of separate determinations performed on duplicate cultures.

to the culture medium (Table 2). However, as with the neuroblastoma cells, addition of ubiquinone to the medium did not produce a significant suppression of HMG-CoA reductase activity, regardless of whether the enzyme was previously induced or repressed by the presence or absence of serum lipoproteins or assayed with or without NaF (Table 2).

There is some evidence indicating that a substantial amount of circulating ubiquinone is associated with LDL *in vivo* (Mabuchi *et al.*, 1981). Although most of the free ubiquinone that we added to the culture medium became associated with serum proteins (see Fig. 2), it was conceivable that the failure of ubiquinone to repress HMG-CoA reductase was due to the ubiquinone not being associated specifically with LDL. Krieger *et al.* (1979) devised a method for reconstituting LDL with ubiquinone and established that the reconstituted lipoprotein can be internalized by cultured fibroblasts. When LDL-ubiquinone was prepared in this way and added to our fibroblast cultures, we failed to observe a consistent decrease in the activity of HMG-CoA reductase (Table 2). In fact, the activity of the enzyme was somewhat elevated, compared with the controls. To confirm that the LDL-ubiquinone was actually taken up by the cells, the residual medium from the cultures assayed for HMG-CoA reductase was added to separate cultures, which were then assayed for ubiquinone after a 24 h incubation period (Table 3). The ubiquinone content of the fibroblasts incubated with the reconstituted LDL-ubiquinone underwent a marked 10–15-fold elevation, similar to that seen in the cells incubated with 'free' ubiquinone (i.e. ubiquinone bound to unspecified serum proteins).

Effect of exogenous ubiquinone on cellular ubiquinone synthesis

Neuroblastoma cells and skin fibroblasts both incorporated [1-¹⁴C]acetate and [2-³H]mevalonolactone into ubiquinone, with the incorporation of the radioactive precursors remaining linear with time for at least 4 h (Fig. 3). On the assumption that a substantial suppression of HMG-CoA reductase activity would result in a decreased flux of carbon from acetyl-CoA into the ubiquinone isoprenoid chains being synthesized within the cell, we measured the rate of incorporation of [1-¹⁴C]acetate into ubiquinone in cells that had been exposed to exogenous ubiquinone for 24 h before the labelling period (Table 4). The data indicate that the incorporation of acetate into ubiquinone was not altered, thereby confirming the results of direct assays of HMG-CoA reductase activity under the same conditions (see Table 2).

Table 4. Effect of exogenous ubiquinone on the rate of cellular ubiquinone synthesis

Neuroblastoma cells were seeded in 75 cm² flasks at a density of 30 000 cells/cm². After 24 h, ubiquinone or an equivalent volume of ethanol was added and the cells were incubated for 24 h. To measure the rate of ubiquinone synthesis, cells were incubated for 4 h with [1-¹⁴C]acetate (2 μCi/ml of medium) or [2-³H]mevalonolactone (2 μCi/ml of medium). Fibroblasts were seeded in 75 cm² flasks at 25 000 cells/cm² and were grown for 48 h before addition of ubiquinone-10. Fibroblasts were incubated with ubiquinone for 24 h and the rate of ubiquinone synthesis was measured by incubating the cells with [1-¹⁴C]acetate (2 μCi/ml of medium) or [2-³H]mevalonolactone (4 μCi/ml of medium) for 4 h at 37 °C. Cellular ubiquinones were extracted and isolated by two-dimensional t.l.c., and the incorporation of radioactivity was determined as described in the Materials and methods section. All values are means (± S.E.M.) of separate determinations performed on three cultures.

Culture conditions		Radioactivity incorporated into ubiquinone (c.p.m./mg of protein)	
		[1- ¹⁴ C]Acetate	[2- ³ H]Mevalonolactone
Neuroblastoma cells	Control	577 ± 50	156 ± 8
	+ Ubiquinone-9 (11.6 μM)	620 ± 21	149 ± 12
Skin fibroblasts	Control	144 ± 7	154 ± 19
	+ Ubiquinone-10 (11.6 μM)	180 ± 3	130 ± 11

To determine whether intracellular accumulation of ubiquinone inhibited ubiquinone synthesis at a point in the pathway beyond the HMG-CoA reductase step, the rate of incorporation of [^3H]mevalonolactone into ubiquinone was measured in neuroblastoma cells and fibroblasts that had been incubated with exogenous ubiquinone for 24 h (Table 4). Since the activity of HMG-CoA reductase was not affected by ubiquinone, it is unlikely that the endogenous mevalonate pools differed substantially in the control and ubiquinone-supplemented cultures. Therefore, the fact that these cultures were nearly identical in their ability to incorporate [^3H]mevalonolactone into cellular ubiquinone (Table 4) suggests that steps in the synthesis of ubiquinone distal to the formation of mevalonate were not affected by the increased uptake of exogenous ubiquinone.

DISCUSSION

The present studies suggest that ubiquinone is not directly involved in the 'feedback' modulation of HMG-CoA reductase in cultured mammalian cells. Our finding that the incorporation of isoprenoid precursors such as [$1\text{-}^{14}\text{C}$]acetate and [^3H]mevalonolactone into ubiquinone was not suppressed by uptake of exogenous ubiquinone is consistent with a previous report that feeding ubiquinone to rats does not decrease the incorporation of *p*-hydroxy[U- ^{14}C]benzaldehyde (a precursor of the quinone ring) into hepatic ubiquinone, despite a marked increase in the ubiquinone concentration of the tissue (Ranganathan & Ramasarma, 1975).

The ubiquinone branch of the isoprenoid-biosynthetic pathway appears to be saturated by a lower concentration of mevalonate-derived prenyl phosphate than that required to saturate the cholesterol branch of the pathway (Gold & Olson, 1966; Ranganathan *et al.*, 1981). Consequently, the rate of ubiquinone synthesis is less sensitive to changes in HMG-CoA reductase activity than is the rate of cholesterol synthesis (Ranganathan *et al.*, 1981). We have shown that neuroblastoma cells can tolerate a 50% decrease in mitochondrial ubiquinone content without impairment of respiratory function (Maltese & Aprille, 1985), and can continue to proliferate when ubiquinone synthesis is inhibited by addition of low concentrations ($\leq 1 \mu\text{M}$) of mevinolin (a competitive inhibitor of HMG-CoA reductase) to the culture medium (Maltese & Sheridan, 1985). Insofar as these observations suggest that mammalian cells do not require precise homeostatic control of endogenous ubiquinone synthesis, they are consistent with the demonstrated absence of end-product repression of HMG-CoA reductase by exogenous ubiquinone.

Several studies have established that complete suppression of mevalonate synthesis results in arrest of cell proliferation and alterations of cell morphology (Habenicht *et al.*, 1980; Schmidt *et al.*, 1982; Quesney-Huneus *et al.*, 1983; Fairbanks *et al.*, 1984; Sinensky & Logel, 1985; Maltese & Sheridan, 1985). However, the molecular events underlying these phenomena remain obscure. Several lines of evidence suggest that the cellular responses to mevalonate deprivation are not mediated solely by depletion of cholesterol (Habenicht *et al.*, 1980; Schmidt *et al.*, 1982; Quesney-Huneus *et al.*, 1983; Fairbanks *et al.*, 1984; Maltese & Sheridan, 1985), dolichol (Schmidt *et al.*, 1982; Maltese & Sheridan, 1985)

or isopentenyladenine (Maltese & Sheridan, 1985; Adair & Brennan, 1986). Our previous finding that addition of ubiquinone to the culture medium was unable to prevent the arrest of growth and neurite formation that occurred in neuroblastoma cells exposed to high concentrations of mevinolin (Maltese & Sheridan, 1985) implied that depletion of ubiquinone also could be ruled out as the primary cause for the cellular responses to mevalonate deprivation. This conclusion is now reinforced by the demonstration that supplementary ubiquinone added to the medium actually augments the intracellular ubiquinone pool in neuroblastoma cells.

Finally, our observations that the ubiquinone concentration in the mitochondria of cultured cells can be either increased, by enriching the medium with ubiquinone, or decreased, by blocking HMG-CoA reductase (Maltese & Aprille, 1985), may prove to be of some practical value in physiological studies focusing on the interactions of ubiquinone with the enzyme complexes of the electron-transport chain.

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