3-Hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured fibroblasts from patients with mevalonate kinase deficiency: differential response to lipid supplied by fetal bovine serum in tissue culture medium

K. M. Gibson,* G. Hoffmann,† A. Schwall,† R. L. Broock,# S. Aramaki,# L. Sweetman,# W. L. Nyhan,# I. K. Brandt, R. S. Wappner,** W. Lehnert,†† and F. H. Trefz##**

Kimberly H. Courtwright and Joseph W. Summers Metabolic Disease Center and Baylor Research Foundation,* Baylor University Medical Center, 3500 Gaston Avenue, Dallas, TX 75246; Universitäts-Kinderklinik Gottingen,† Schwerpunkt Neuropadiatrie, Gottingen, Federal Republic of Germany; Department of Pediatrics, Division of Biochemical Genetics,# University of California San Diego, La Jolla, CA; Department of Pediatrics and Medical Genetics,** Indiana University and James Whitcomb Riley Hospital for Children, Indianapolis, IN; Universitäts-Kinderklinik,†† Klinikum der Albert Ludwigs Universität, Freiburg, Federal Republic of Germany; and Universitäts-Kinderklinik,## Heidelberg, Federal Republic of Germany

Abstract 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity was measured in extracts of cultured fibroblasts derived from patients with mevalonate kinase deficiency (MKD). For six patients studied, the mean activity of 63.3 ± 41.1 pmol/min-mg protein (± 1 SD, range 37.7–146.2) was significantly higher than the mean value in three control fibroblast lines of 11.1 ± 3.5 (± 1 SD, range 8.0–14.9). These values were obtained using cells subcultured in medium supplemented with 10% fetal bovine serum (FBS) 21 h prior to assay. When cells were deprived of cholesterol by subculturing for 21 h in delipidated FBS, the mean value for patient cells was increased to 230.8 ± 78.5 pmol/min-mg protein (range 130.9–333.8) as compared to 109.5 ± 47.1 (range 78.0–163.6) for controls. The activity of HMG-CoA synthase in extracts of fibroblasts derived from the patients was not elevated. The mevalonic acid concentration in the surrounding culture medium was assessed by stable isotope dilution assay. For five patients, the mean concentration in medium containing FBS was 0.92 ± 0.37 μM (± 1 SD, range 0.46–1.48) in contrast to 1.24 ± 0.83 μM (range 0.46–2.54) for cells subcultured in delipidated FBS. The mean value for three control fibroblast lines was 0.22 ± 0.12 μM (± 1 SD, range 0.11–0.35) for cells subcultured in FBS as compared to 0.01 ± 0.01 μM (range 0.0–0.01 μM) for cells subcultured in delipidated FBS. The activity of the cholesterol biosynthetic pathway, assessed by monitoring [^{14}C]acetate incorporation into cholesterol in fibroblast monolayers for cells subcultured in both FBS and delipidated FBS, was comparable between patient and control cell lines despite the finding of virtually undetectable mevalonate kinase activity in fibroblast extracts of the patients. The absolute levels of control mevalonate kinase activity were comparable for cells subcultured in FBS or delipidated FBS. These data suggest that MKD cells compensate for diminished mevalonate kinase activity by regulating HMG-CoA reductase upward to increase intracellular concentration of mevalonic acid and permit the normal process of sterol synthesis.—**Gibson, K. M., G. Hoffmann, A. Schwall, R. L. Broock, S. Aramaki, L. Sweetman, W. L. Nyhan, I. K. Brandt, R. S. Wappner, W. Lehnert, and F. H. Trefz.** 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity in cultured fibroblasts from patients with mevalonate kinase deficiency: differential response to lipid supplied by fetal bovine serum in tissue culture medium. *J. Lipid Res.* 1990. **31:** 515–521.

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Mevalonate kinase (ATP: mevalonate-5-phosphotransferase, EC 2.7.1.36) deficiency is a rare, inherited defect in the biosynthesis of cholesterol and nonsterol isoprenes (1–3, **Fig. 1**). The disorder is characterized by the urinary excretion of mevalonic acid, an intermediate in the cholesterol biosynthetic pathway. Mevalonate kinase deficiency (MKD) represents the interruption of a biosynthetic pathway, while most inherited disorders involve catabolic pathways.

Cardinal clinical manifestations in six cases of MKD have included anemia, hypotonia, psychomotor retardation, ataxia, and enteritis (1–5). In three cases, the activi-

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; MKD, mevalonate kinase deficiency; FBS, fetal bovine serum; GLC-MS, gas-liquid chromatography-mass spectrometry; LDL, low density lipoprotein

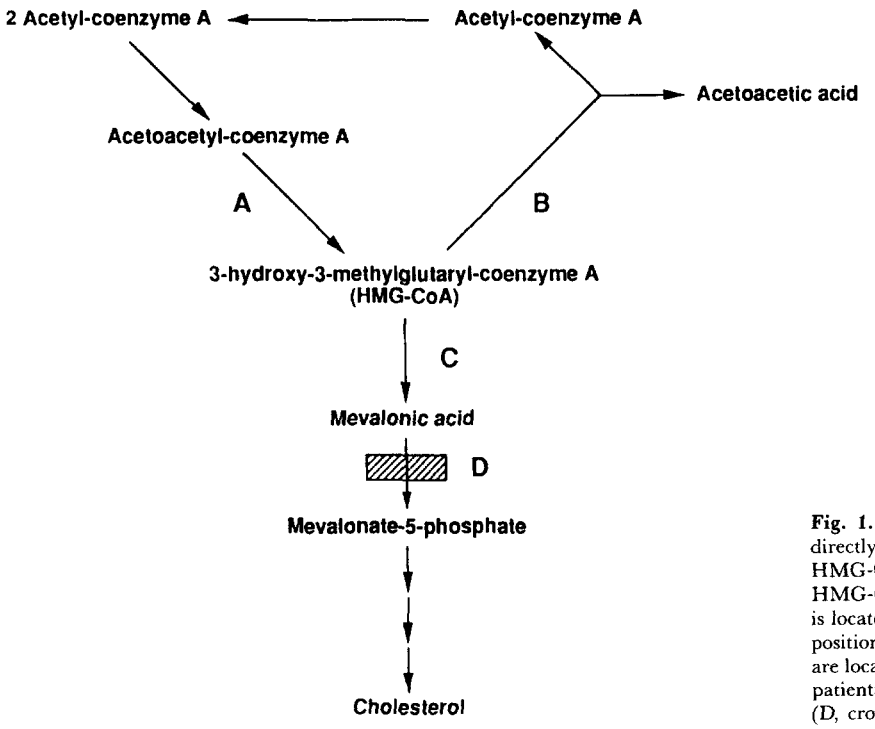

Fig. 1. The HMG-CoA cycle. Three enzymes are directly involved in the metabolism of HMG-CoA: HMG-CoA synthase (A), HMG-CoA lyase (B), and HMG-CoA reductase (C). Of these reactions, only B is located within the mitochondrion, occupying a key position in the ketogenic pathway. All other reactions are located within the cytosol. The site of the block in patients with mevalonic aciduria is mevalonate kinase (D, cross-hatched box).

ty of creatine kinase was substantially elevated, and in all cases the serum cholesterol concentrations were normal or only slightly decreased. Mevalonic acid excretion in the urine of patients has been widely variable, ranging from 1 to 56 mol/mol creatinine (control values 0.2–0.3 mmol/mol creatinine) (6). Heterogeneity in phenotypic manifestations should be defined as more patients with this disorder are described.

Manifestations of this disease could result from a shortage of a number of biologically important products, including cholesterol, bile acids, steroid hormones, dolichol, ubiquinone, heme A, and isopentenyl-t-RNA. It is also possible that pathophysiology is directly related to increased intracellular levels of mevalonic acid, as suggested by Keller and Simonet (7). Measurement of serum concentrations of cholesterol, on the other hand, have been normal or only slightly reduced (1–5). Concomitantly, the urinary excretion of mevalonic acid is massive. These observations led us to hypothesize that HMG-CoA reductase regulated upward might at least partially compensate for the fundamental metabolic lesion in the cell. We have investigated this possibility by measuring HMG-CoA reductase activity in cultured fibroblasts obtained from six patients and three control individuals. Concentrations of mevalonic acid in tissue culture medium were quantified by means of stable isotope dilution assay with gas–liquid chromatography–mass spectrometry (GLC-MS). The data indicate that the MKD in cultured cells and presumably in vivo is balanced by increased activity of HMG-CoA reductase.

METHODS

D_3-Mevalonic acid was prepared by custom synthesis (MSD Isotopes, Pointe-Claire, Dorval, Quebec). 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA, [3-^{14}C]glutaryl), R,S-[2-^{14}C]mevalonolactone, and sodium [2-^{14}C]acetate were purchased from New England Nuclear (Boston, MA). Human low density lipoprotein (LDL), prepared by density centrifugation, was a gift from Dr. Daniel Steinberg. All other reagents were of the highest commercial purity available.

The methodology for preparation of lipid-poor fetal bovine serum (delipidated FBS) was supplied by Drs. Luskey, Brown, and Goldstein of the University of Texas Southwestern Medical Center at Dallas. In essence, 20 g thixotropic gel powder (Cab-o-sil, Kodak) was added to 1 liter FBS and stirred overnight at 4°C. The mixture was then centrifuged at 15,000 rpm for 1 h at 4°C and the supernatant fluid was sequentially filtered through Buchner 50F filter paper and Nalgene units with decreasing pore sizes of 0.45 and 0.20 μm.

Fibroblast monolayers were maintained in Coon's F-12 medium supplemented with 10% FBS, glutamine, penicillin, and streptomycin. To investigate the effect of LDL on HMG-CoA reductase and mevalonate kinase activity, the following methodology was used. On day 1, 100-mm plates were seeded with 5×10^5 cells/plate. Growth was maintained in an atmosphere of 95% air: 5% CO_2. On day 3, the media was replaced with fresh media. At day 6, the medium was removed, the attached cells were

washed twice with phosphate-buffered saline (PBS), and replaced with fresh Coon's F-12 medium supplemented with either 10% FBS or 5% delipidated FBS. On day 7 (after 21 h incubation), the attached cells were washed with 50 mM Tris-Cl, 0.15 M NaCl, pH 7.4 (buffer A), and harvested by scraping into 3 ml of buffer A. Fibroblasts were pelleted by centrifugation and washed again by gentle resuspension in buffer A. The pelleted cells were either kept at −70°C prior to enzyme analysis or assayed immediately. Fibroblasts from two 100-mm plates were combined and resuspended in 0.15 ml 50 mM KH_2PO_4, pH 7.4, supplemented with 5 mM DTT, 5 mM EDTA, 0.25% Kyro-EOB, and 0.2 M KCl (8). Total cellular protein was estimated from aliquots of this resuspension by the method of Lowry et al. (9). In most cases (with the exception of patient BVL) all plates had reached confluency by day 7 (approximately 3–4 × 10^6 cells/plate). HMG-CoA reductase and mevalonate kinase activities were estimated as previously described (3, 8).

Quantitative measurements of mevalonic acid concentrations in tissue culture medium were preformed by GLC-MS. For these studies, 50 nmol of D_3-mevalonic acid was added to 9-ml volumes of tissue culture medium (FBS or dilipidated FBS) in which cells had been subcultured for 21 h. After being transferred to 15 ml conical tubes, the samples were deproteinized by adding 1 ml 4.2 M $HClO_4$ and kept on ice for 10 min. After centrifugation, the supernatant fluid was neutralized with 0.7 ml 6 N KOH, the insoluble $KClO_4$ was removed by centrifugation, and the resulting supernatant was lyophilized to dryness. The same procedure was followed for samples prepared with known amounts of mevalonic acid not containing deuterium (D_0) (0, 1, 3, 5, 10, 25, 50, and 100 nmol) for the standard curve. The dried samples were acidified with 0.1 ml of concentrated H_2SO_4 and kept on ice for 30 min to ensure lactonization of mevalonic acid.

Mevalonic acid (D_0,D_3) was isolated from samples by liquid partition chromatography. Using a portable sieve shaker, silicic acid (0.2-kg aliquots, refined and acid washed, Sigma catalogue S-8763) was sieved through sequentially decreasing pore sizes of 150, 75, and 38 μm for 30 min. Silicic acid retained in the 38-μm sieve was dried overnight at 130°C. For the preparation of sample silicic acid, a slurry consisting of 50 g dried silicic acid and 120 ml 40% 2-methylbutan-2-ol-60% chloroform was poured into an Omni preparative glass column (500 mm × 25 mm i.d., Rainin Instrument Co., Woburn, MA) and eluted for 90 min with the same solvent mixture at a flow rate of 7.5 ml/min. Solvent was removed from sample silicic acid by drying in an oven at 130°C for 48 h. Column silicic acid was prepared by hydration of 92 g sample silicic acid with 42 ml 0.1 N H_2SO_4. For isolation of mevalonic acid, the acidified samples and standards were adsorbed onto 0.75 g sample silicic acid. For each analysis, a new column was prepared by introduction of 1 g column silicic acid into an Omni analytical column (250 mm × 6.6 mm i.d.). The sample, previously adsorbed onto sample silicic acid, was then placed on top of the bed of column silicic acid, the column was filled with 3% 2-methylbutan-2-ol in chloroform, and the column was eluted with this solvent for 9 min at a flow rate of 2.5 ml/min. Eluants were collected in 22-ml glass scintillation vials containing 2 ml of 10 mM NaOH in methanol and rosolic acid as an acid–base indicator. After chromatography, solvents were evaporated under a gentle nitrogen stream in a fume hood. The dried samples were then acidified with 0.2 ml of 0.5 N HCOOH prior to further analyses.

The ratios of D_0/D_3 mevalonic acid were determined by GLC-MS with a Finnegan 4021 quadrapole GLC-MS with INCOS data system. The GLC conditions included: injector temperature 200°C; separator temperature 300°C; column temperature isothermally operated at 155°C; He carrier gas 16.5 ml/min. The GLC column was a DB Wax Megabore column (0.53 mm × 15 m, J & W Scientific, Rancho Cordova, CA). The GLC column was directly interfaced to the MS ion source inlet. Two μl of each sample was injected. MS conditions were: ionizer temperature 250°C; collision energy 70 eV. Chemical ionization was achieved with 0.06 Torr ammonia as the reagent gas introduced at the ionizer inlet. Selected ion monitoring (SIM) was performed at m/e 113 and 116 $(M + H-H_2O)+$ for D_0 and D_3 mevalonic acid, respectively, using 100 ms dwell time for each m/e. SIM peak areas were integrated by the computer after operator selection of the baseline points. Standard curves were calculated by linear regression. For standard curve preparation, the ratio of areas of D_0/D_3 were plotted on the ordinate as a function of known amounts of mevalonic D_0 (0–100 nmol) on the abscissa; r value was routinely greater than 0.999.

HMG-CoA synthase was assayed as previously described (10). Incorporation of [2-^{14}C]acetate into cholesterol in intact fibroblast monolayers was performed as previously described (11). Statistical analyses were performed using the Student's two-tailed t test (12).

RESULTS

The activities of HMG-CoA reductase and mevalonate kinase in extracts of cultured fibroblasts of patients and controls are displayed in **Table 1**. The mean activity of HMG-CoA reductase was 63.3 pmol/min-mg protein for the patient fibroblasts grown in untreated FBS, a value approximately six times higher than that of 11.1 pmol/min-mg protein for control fibroblasts grown under identical conditions. When fibroblasts were grown in delip-

TABLE 1. HMG-CoA reductase and mevalonate kinase activities in fibroblast extracts, and concentrations of mevalonic acid in tissue culture medium, derived from controls and six patients with mevalonic aciduria cultured in untreated and lipid-depleted FBS

Subject	HMG-CoA Reductase		Mevalonate Kinase		Mevalonic Acid in Culture Medium	
	FBS +	FBS −	FBS +	FBS −	FBS +	FBS −
	pmol/min/mg protein		*nmol/min/mg protein*		*μmol/l*	
Patient (ZW)	46.8, 72.6 46.7, 40.0	273.5, 333.5 108.7, 144.9	0, 0	0, 0	0.84, 1.83 1.76	0.55, 1.74 1.63
Patient (WS)	44.1, 62.0	102.2, 255.0	0, 0	0, 0	0.89, 0.68	0.47, 0.45
Patient (BVL)	85.9, 206.4	205.4, 422.5	0.10, 0	0, 0	0.94, 0.72	0.85, 1.74
Patient (JB)	51.5, 52.4	126.3, 135.5	0	0, 0	0.49, 0.42	0.59
Patient (BK)[a]	29.8, 26.6 56.8	315.3, 315.9 370.2	0, 0	0, 0	0.54, 1.50	1.22, 3.86
Patient (PK)[a]	30.7, 48.0	345.2, 79.6	0	0		
Mean patient[b] (± 1 SD)	63.3 ± 41.1	230.8 ± 78.5	0.01 ± 0.02	0	0.92 ± 0.37	1.24 ± 0.83
Control 1	12.8, 8.1	84.5, 89.1	2.35, 0.78	1.21, 0.38	0.37, 0	0, 0
Control 2	13.9, 5.0 5.1	58.5, 59.2 116.3	2.94	2.03	0.21, 0	0, 0.01
Control 3	17.4, 18.0 9.4	178.0, 133.9 178.9	1.11, 2.83 2.95	1.08, 4.16 3.22	0.80, 0.26 0	0.03, 0 0
Mean control[b] (± 1 SD)	11.1 ± 3.5	109.5 ± 47.1	2.27 ± 0.69	1.88 ± 1.02	0.22 ± 0.12	0.01 ± 0.01[c]

Conditions of culture and enzyme assay are described in Methods. Each enzyme value represents a single determination for cells harvested from one petri dish; similarly, each media value is a single determination for the media surrounding the attached cells of one petri dish. FBS + and FBS − represent untreated and delipidated FBS, respectively.

[a]Patients BK and PK are siblings (female and male, respectively).

[b]Mean values are calculated using the mean value of all determinations for individual cell lines, i.e., the mean of means.

[c]For comparison, concentrations of mevalonic acid in fresh culture medium supplemented with 10% FBS ranged from 0.01 to 0.04 μmol/l.

idated FBS for 21 h, the mean value was 230.8 pmol/min-mg protein for the patients as compared to 109.5 pmol/min-mg protein for controls. The induction ratio (HMG-CoA reductase in untreated FBS/HMG-CoA reductase in delipidated FBS) was 9.9 for control in contrast to 3.6 for the patients. This significantly lower patient value reflects the high level of unsuppressed HMG-CoA reductase activity in the cells cultured in untreated FBS containing cholesterol. The ability of the patient fibroblasts to further increase HMG-CoA reductase in response to delipidated FBS suggests that the activity of this key regulatory enzyme in the cholesterol biosynthetic pathway is regulated in a fashion similar to that demonstrated by normal cells (13).

Despite the fact that patient and control fibroblasts were maintained under identical culture conditions for studies involving untreated and delipidated FBS, there was considerable scatter even within individual fibroblast lines for replicate studies (Table 1). This may reflect day-to-day variation in cell harvesting or assay methodology, or it may simply reflect the variation of the enzymes studied. In spite of this, the mean values for HMG-CoA reductase activity for fibroblasts subcultured in untreated FBS (FBS +) or delipidated FBS (FBS −) were significantly different between patients and controls ($P<0.05$ for both growth conditions).

Mevalonate kinase activity was essentially undetectable in fibroblast extracts derived from all six patients (Table 1). The activity of this enzyme was also studied in control fibroblasts grown in untreated FBS and delipidated FBS. The levels of activity were 2.27 ± 0.69 for cells grown in untreated FBS and 1.88 ± 1.02 for cells grown in delipidated FBS, revealing no significant difference.

Mevalonic acid concentrations in tissue culture medium were 0.92 μM and 1.24 μM for patient cells grown in untreated and delipidated FBS, respectively, whereas for control cells, the values were 0.22 and 0.01 in untreated and delipidated FBS, respectively, Again, there was substantial scatter in the values for individual cell lines, with a significant difference between patient and control mean metabolite levels occurring only under conditions of subculture in FBS − . These data indicate that the patient cells maintain a high activity of HMG-CoA reductase despite differences in culture conditions, and that this high activity is accompanied by an efflux of mevalonic acid into the surrounding tissue culture medium. On the other hand, control cells appear to excrete a smaller, but still quantifiable, level of mevalonic acid into the untreated FBS medium. This value is near zero when the control cells are grown with delipidated FBS, suggesting that mevalonate is conserved intracellularly to ensure the production of cholesterol and nonsterol isoprenes.

This level of excretion appeared relatively constant in patient cells, despite an approximately fourfold increase in HMG-CoA reductase activity when cells were switched from untreated FBS to delipidated FBS (Table 1). This finding might reflect an increased flux of mevalonic acid through the mevalonate kinase step, in the patients' cells, allowing for increased sterol synthesis. In response to upward regulated HMG-CoA reductase activity, this would explain why mevalonic acid excretion into the culture medium was not significantly increased when MKD cells were switched to lipid-poor FBS.

We attempted to ascertain whether the high level of HMG-CoA reductase in the patients' cells could be suppressed by adding known quantities of purified LDL-cholesterol (**Table 2**). For these studies, patient and control cells were subcultured in medium replete with delipidated FBS and delipidated FBS containing 5, 10, and 20 μg/ml LDL-cholesterol (Table 2). In both patients and controls, the level of HMG-CoA reductase in cells grown in untreated FBS (C, Table 2) in most cases approximated that of cells subcultured in delipidated FBS with 20 μg/ml LDL-cholesterol. However, in the three patient cells lines studied, the absolute level of HMG-CoA reductase activity was significantly higher than controls under both sets of conditions. Patients BK and PK (siblings) showed a higher level of inhibition in HMG-CoA reductase activity in the presence of 20 μg LDL/ml culture medium (approximately 85–95%, similar to that of control fibroblast cell lines), whereas patient ZW showed a lower (60–70%) degree of inhibition (Table 2) This finding may reflect an experimental error in single determinations. On the other hand, HMG-CoA reductase activity for the siblings' cell lines grown in delipidated FBS was threefold higher than that of patient ZW grown under identical conditions. This may account for the observed lower percentage of inhibition in the cells of patient ZW.

TABLE 2. Fibroblast HMG-CoA reductase activity as a function of tissue culture condition

Subject	HMG-CoA Reductase				
	C	D	5	10	20
	pmol/min/mg protein				
Control 2	5.1	116.3	15.8	8.4	6.9
Control 3	9.4	178.9	23.0	16.1	12.0
Patient ZW[a]	40.0	144.9	190.4	78.1	81.3
Patient ZW	46.7	108.7	63.3	41.8	33.0
Patient BK	56.8	370.2	171.1	91.8	58.0
Patient PK	30.7	345.2	54.4	22.9	18.1

Abbreviations: C, control (cells grown in untreated FBS); D, delipidated (cells grown in delipidated FBS); 5, 5 μg LDL/ml culture medium (delipidated FBS); 10, 10 μg LDL/ml culture medium (delipidated FBS); 20, 20 μg LDL/ml culture medium (delipidated FBS). Growth periods were 21 h.

[a]Studies for patient ZW were carried out on two separate occasions.

TABLE 3. Incorporation of [2-^{14}C]acetate into cholesterol by intact fibroblast monolayers derived from patients with mevalonate kinase deficiency, family members, and control individuals

Subject	FBS +	FBS −
	dpm [^{14}C]cholesterol (μg protein)$^{-1}$	
Control	54 ± 45 (7–150, n = 4)	85 ± 75 (7–150, n = 4)
Family members	52 ± 43 ($P > 0.5$) (8–151, n = 7)	154 ± 84 ($P > 0.3$) (47–282, n = 6)
Patients	15 ± 9 ($P > 0.2$) (6–33, n = 5)	109 ± 59 ($P > 0.5$) (74–177, n = 3)

Incorporation studies were performed as previously described (10). Incubations were carried out for 24 h, and each cell line was assayed in duplicate on two separate occasions. All values are the mean ± 1 standard deviation, with range in parentheses; n values represent the number of cell lines studied.

Despite a severe deficiency of mevalonate kinase in extracts of fibroblasts derived from the patients, there was still significant activity for the cholesterol biosynthetic pathway, assessed by measuring the incorporation of [2-^{14}C]acetate into cholesterol in intact fibroblast monolayers (**Table 3**). For cells grown with FBS, the mean patient activity of 15 dpm [^{14}C]cholesterol/μg protein was approximately 28% of the same activity in fibroblasts derived from controls and family members, although the ranges of values were overlapping for all cell types and the means were not significantly different ($P > 0.2$). Similar results were obtained for studies in delipidated FBS. These data indicate that MKD cells, when stressed by growth in cholesterol-poor media, are capable of synthesizing cholesterol at a normal rate, an interesting result in view of the almost complete absence of mevalonate kinase activity in patient fibroblast extracts. This finding may reflect an inherent instability of the mutant enzyme in tissue extracts, with the defect being only partial in the intact cell.

In further regulatory studies, the activity of HMG-CoA synthase in extracts of fibroblasts derived from patients and controls was assessed. The values were 252 ± 105 (± 1 SD, range 111–359) pmol/min per mg protein for five control fibroblast lines in comparison with 170 ± 68 (range 51–210) pmol/min per mg protein for five patient cell lines, indicating normal synthase activity. For HMG-CoA lyase, the values were 662 ± 174 (range 516–854) pmol/min per mg protein for three control fibroblast lines in comparison with 1140 ± 579 (range 438–1835) pmol/min per mg protein for five patient cell lines, suggesting normal lyase activity. These data indicate that only the reductase of the HMG-CoA cycle enzymes was regulated upward in the patient cell lines. This suggested a decrease in feedback inhibition by cholesterol and/or other nonsterol isoprenes.

DISCUSSION

The activity and regulation of HMG-CoA reductase in cultured fibroblasts from patients with MKD have not previously been studied. Hoffmann et al. (11) indirectly assessed HMG-CoA reductase activity by assessing mevalonic acid concentrations in tissue culture medium, in a fashion similar to the procedure described in the present report, although direct enzyme assays were not reported. The increased levels of mevalonic acid in culture medium surrounding the cells of patients with MKD documented in the present report are in excellent agreement with earlier preliminary results (11). Keller and Simonet (7) assessed HMG-CoA reductase activity in isolated liver microsomes from a MKD affected fetus (2) and found normal activity. These investigators concluded, however, that reductase activity was artefactually decreased, probably due to autolysis, since there was a large increase in liver mevalonic acid concentrations previously documented (840–1120 μmol/kg vs 2 μmol/kg in control fetal liver) (14). A striking feature of MKD is the enormous amount of mevalonic acid excreted in the urine, suggesting that this disorder may be partially offset by an increase in activity of HMG-CoA reductase. We found that this was the case when the activity of the enzyme in tissue extracts of the patients' cells was more than sixfold higher than that of control fibroblasts. Quantification by stable-isotope dilution assay of mevalonic acid levels indicated that the cells of the patients accumulated a significant amount of mevalonic acid both in the presence and absence of LDL-cholesterol. Control cells appeared to utilize mevalonic acid when grown in delipidated FBS, ensuring the production of cholesterol and other nonsterol isoprenes.

Several interrelated processes may occur in the cells of the patients. Defective mevalonate kinase may have altered kinetic properties, such as elevated K_m, which permit the enzyme to function at a reasonable velocity in the presence of a significantly higher mevalonic acid concentration. Increased activity of HMG-CoA reductase could be interpreted as a cellular response to this need. However, if this were the case, an elevation in the activity of HMG-CoA synthase might be expected, which we found not to be evident. More likely, elevated activity of the reductase may be a function of decreased feedback inhibition by cholesterol and other nonsterol isoprenes. Since most, if not all, of these patients have normal-to-near-normal concentrations of serum cholesterol, we might attribute any decrease in the level of feedback inhibition to nonsterol products, such as dolichol, ubiquinone, heme A, or isopentenyl-t-RNA. Any or all of these factors may be simultaneously involved. It is noteworthy, however, that Keller and Simonet (7) found levels of cholesterol, dolichol pyrophosphate, and squalene that were normal-to-near-normal in extracts of liver obtained from a fetus with MKD. This finding led these investigators to suggest that increased concentrations of mevalonic acid in tissues and body fluids of patients might be at least partially responsible for the underlying pathophysiology.

The cells of the patients with MKD demonstrated a substantial level of unsuppressed HMG-CoA reductase activity. However, the regulatory mechanism of this enzyme in the mutant cells appeared to be identical to that of control cells. HMG-CoA reductase activity, even in the presence of 20 μg/ml of LDL-cholesterol in the culture medium, was still higher in patient cells than in control cells. This level of LDL-cholesterol has previously been reported to achieve almost complete down-regulation of HMG-CoA reductase activity in control cells (15, 16). On the other hand, the percent of inhibition (i.e., HMG-CoA reductase activity in delipidated FBS + 20 μg LDL-cholesterol divided by HMG-CoA reductase activity in delipidated FBS alone) by 20 μg LDL-cholesterol/ml appeared quite similar at approximately 60–95% for both control and patient cells lines. These data imply that the degree of inhibition is the same in both cell types even though basal activities (defined as those expressed in cells grown in untreated 10% FBS) are higher in patient cells.

It is of interest that the ratio of HMG-CoA reductase activity to mevalonic acid concentration was relatively constant for cells grown in untreated FBS (69:1 and 50:1, respectively, for patient and control). This observation might argue against elevated HMG-CoA reductase as a mechanism to ensure increased amounts of mevalonic acid to compensate for decreased mevalonate kinase enzyme levels or decreased enzyme affinity. When considered in conjunction with the finding of normal HMG-CoA synthase activity in the patients' cells, these data would support an argument in favor of decreased end-product feedback inhibition as the cause of upward regulated HMG-CoA reductase in the patients' fibroblasts.

Removing lipid from tissue culture medium had no effect on mevalonate kinase activity in control cells, suggesting that fibroblast mevalonate kinase was not subject to regulation by cholesterol. Our findings contrast those reported by Mitchell and Avigan (17) who found that mevalonate kinase activity increased twofold upon incubation of a control fibroblast cell line in lipid-depleted FBS. In the present study, replicate determinations of mevalonate kinase activity were performed using three control fibroblast cell lines. Moreover, we have observed that enzyme determinations performed with fibroblast extract as the source of enzyme (Table 1) can readily vary by twofold for measurements employing sequential subcultures. It remains possible that different preparations of lipid-depleted FBS (produced via solvent extraction or thixotropic gel powder treatment) may be responsible for the variation in results described above.

Without doubt, a major finding in the present report is the ability of MKD cell lines to synthesize sterols at a

normal rate in intact cells despite a finding of deficient mevalonate kinase activity in cell extracts. It is certainly possible that low levels of residual mevalonate kinase activity may not be detected by the assay we used. Mutant mevalonate kinase also may be inherently unstable, and may be even more so when cell-free extracts are prepared. It is intriguing to speculate on an alternate pathway for sterol formation from acetate which would bypass the mevalonate kinase step. Such a pathway might correspond to that of the shunt pathway linking leucine metabolism to the pathway of sterol formation (18).

Studies on cells that lack LDL-receptor activity from patients with familial hypercholesterolemia (19) have elegantly demonstrated the utility of studies on cultured fibroblasts in elucidating mechanisms of cholesterol biosynthesis. The cultured cell model also has been useful in the study of MKD. Mevalonic acid accumulates intracellularly in response to the metabolic defect and the decreased feedback inhibition. It then diffuses out of the cell and accumulates into the culture medium. This is a model for what goes on in vivo, where accumulated mevalonic acid enters the circulation and is cleared by the kidney. Other questions concerning the regulation of cholesterol and nonsterol isoprene biosynthesis in MKD may be effectively investigated using the cultured cell model, and the MKD cell line may be of particular utility.

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