Randerath, K. (1967), *Anal. Biochem. 21*, 480.
Randerath, K., and Randerath, E. (1967), *J. Chromatog. 31*, 500.
Rubin, I. B., Kelmers, A. D., and Goldstein, G. (1967), *Anal. Biochem. 20*, 533.
Sarin, P. S., and Zamecnik, P. C. (1965), *Biochem. Biophys. Res. Commun. 20*, 400.
Schofield, P., and Zamecnik, P. C. (1968), *Biochim. Biophys. Acta 155*, 410.
Scott, J. F., and Schofield, P. (1969), *Proc. Natl. Acad. Sci. U. S. 64*, 931.
Seno, T., Kobayashi, M., and Nishimura, S. (1968), *Biochim. Biophys. Acta 169*, 80.
Stern, R., Zutra, L. E., and Littauer, U. Z. (1969), *Biochemistry 8*, 313.
Trupin, J., Dickerman, H., Nirenberg, M., and Weissbach, H. (1966), *Biochem. Biophys. Res. Commun. 24*, 50.
Weiss, J. F., and Kelmers, A. D. (1967), *Biochemistry 6*, 27.
Weiss, J. F., Pearson, R. L., and Kelmers, A. D. (1968), *Biochemistry 7*, 3479.

Solubilization and Purification of β-Hydroxy-β-methylglutaryl Coenzyme A Reductase from Rat Liver*

Takashi Kawachi† and Harry Rudney‡

ABSTRACT: β-Hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase from the rat liver microsomal fraction has been highly purified. This is the first such preparation of HMGCoA reductase obtained from mammalian sources. Solubilization of the enzyme was attained by treatment of the microsomal fraction with sodium deoxycholate and subsequent purification proceeded by standard methods. The molecular weight determined by gel filtration and centrifugation techniques ranged from 217,000 to 226,000. The enzyme showed many properties similar to the HMGCoA reductase of yeast (Kirtley, M. E., and Rudney, H. (1967), *Biochemistry 6*, 230). These were Michaelis constants, inhibition by acyl-CoA derivatives and CoA, a strong requirement for thiols, and inhibition by *o*-phenanthroline. It was also very sensitive to sulfhydryl reagents. Deoxycholate and digitonin inhibited the enzyme while cholesterol had no effect. Some metal ions, *e.g.*, Fe^{2+}, and Fe^{3+}, were strongly inhibitory.

Many agents, *e.g.*, dithiothreitol, deoxycholate, and ethylene diaminetetraacetate, showed a differential effect on the crude and purified preparations. These are discussed in relation to possible control mechanisms.

The incorporation of acetate into cholesterol in the liver can be drastically reduced by cholesterol feeding and by fasting (Gould, 1951; Langdon and Bloch, 1953; Tomkins *et al.*, 1953; Bucher and McGarrahan, 1956) while extrahepatic tissues are relatively unaffected by these regimes (Dietschy and Siperstein, 1967).

The inhibition of hepatic cholesterogenesis observed with mevalonate as substrate was minor compared with the inhibition from acetate (Bucher *et al.*, 1959). These observations led to the conclusion that the major part of the physiological regulation of cholesterol biosynthesis lies at the step between HMGCoA and mevalonate (Bucher, 1959; Siperstein and Guest, 1959; Bucher *et al.*, 1960; Siperstein and Fagan, 1966).

The enzyme catalyzing the reduction of HMGCoA to mevalonate (HMGCoA reductase–mevalonate:NADP-oxidoreductase (acylating-CoA), EC 1.1.134) has been purified by Durr and Rudney (1960), and Kirtley and Rudney (1967), and by Knappe *et al.* (1959) from yeast. Recently a report by Linn (1967) on the solubilization of HMGCoA reductase from extracts of acetone powders of rat liver microsomes appeared. Even without further purification, Linn was able to show clearly that the effect of cholesterol feeding appeared to be directly expressed as a decrease in the activity of the HMG-CoA reductase in these preparations. We attempted to use the acetone powder extracts of Linn as a basis for further purification, but although these extracts were active we were unable to obtain truly soluble preparations of the reductase using his procedure. In this paper we describe a method for solubilizing the HMGCoA reductase using deoxycholate. From this preparation we were able to purify the enzyme to near homogeneity and examine several of the properties of this system. Siperstein (1965) has indicated that deoxycholate could solubilize preparations of HMGCoA from rat liver microsomes, but did not describe any subsequent purification.

* From the Department of Biological Chemistry, College of Medicine, University of Cincinnati, Cincinnati, Ohio 45219. *Received October 13, 1969.* Supported by grants from the Life Insurance Medical Research Fund and the National Institute for Arthritis and Metabolic Diseases Grant No. AM-12402.

† Present address: National Cancer Center Research Institute, Tokyo, Japan.

‡ Author to whom inquiries concerning this paper should be addressed.

[1] Abbreviations used are: HMGCoA, β-hydroxy-β-methylglutaryl coenzyme A; MVA, mevalonic acid.

Materials

TPNH, TPN, and glucose 6-phosphate were obtained from Sigma Chemical Co. Glucose 6-phosphate dehydrogenase was obtained from Boehringer und Soehne. CoASH was from Pabst Laboratories. [3-^{14}C]HMG was purchased from New England Nuclear. [3-^{14}C]HMGCoA was synthesized from HMG-anhydride by the procedure of Hilz *et al.* (1958). The anhydride was recrystallized twice from benzene and melted at 101–102°. MVA was obtained from Nutritional Biochemical Corp; dithiothreitol from Calbiochem; DEAE-cellulose (DE-11) from Whatman Co.;[2] Dowex 1-X10, 200–400 mesh and AG-50W-X4 (200–400 mesh), a cation exchanger, from Bio-Rad Laboratories; Celite 500 from Johns Manville Co.; and Sephadex G-200 from the Pharmacia Co. Sodium deoxycholate was special enzyme grade from Mann Research Laboratories. Hydroxylapatite was prepared as described by Levin (1962).

Methods

Assay of HMGCoA Reductase. The assay is based on the procedure developed by White and Rudney (1970) to study the incorporation of various precursors into mevalonic acid. This method takes advantage of the fact that at acid pH nonpolar mevalonolactone passes through a Dowex 1 formate column, while all ions are retained, *e.g.*, HMG and acetate. In those cases where acetate was the substrate it was observed that commercial samples of [^{14}C]acetic acid contained a nonpolar substance which contaminated the mevalonolactone fraction. If the mevalonolactone was rechromatographed on Celite columns, the contaminant was removed. In all cases described in this paper, the substrate was [^{14}C]HMGCoA which did not contain a contaminant of this nature. However, in the assay of crude preparations, the rechromatography on Celite was routinely carried out to insure that contamination did not inadvertently occur. The reaction mixture contained 10 μmoles of TPN, 4 μmoles of sodium glucose 6-phosphate, 0.7 unit of glucose 6-phosphate dehydrogenase, 3.5 μmoles of dithiothreitol, 300 nmoles (150 nCi) of [3-^{14}C]HMGCoA, 100 μmoles of EDTA, 10 μmoles of MVA, 1 mole of potassium phosphate buffer (pH 6.9), and enzymes in a final volume of 5.0 ml. For experiments on inhibition and activation, the TPNH-generating system was replaced by 3 μmoles of TPNH. The mixture was incubated for 60 min at 37° and then the reaction was stopped by the addition of 0.8 ml of 8 N sodium hydroxide; 140 μmoles of MVA was added as carrier and the mixture was allowed to stand for 60 min. It was acidified to pH 2.0 by the addition of sulfuric acid with bromothymol blue as indicator. Precipitated protein was removed by centrifugation and the supernatant solution was dried by a stream of air at room temperature. The residue was taken up in 2 ml of water, neutralized with 1 N sodium hydroxide, and extracted with 20 ml of ethanol. The extract was evaporated to dryness by a stream of air at room temperature, and then dissolved in 5 ml of water. AG-50W (2 g; H form) was suspended in the solution and the mixture was centrifuged. The supernatant solution was applied to a Dowex 1 formate column (1.0 × 15 cm) which was eluted with 25 ml of water. The water eluate was evaporated to dryness. The residue was dissolved in 1 ml of 3 N sulfuric acid, mixed with 2 g of Celite, and then applied to the top of the Celite column prepared by mixing 10 g of Celite 500 with 5 ml of 0.2 N H_2SO_4. The column was eluted with chloroform equilibrated with 0.2 N H_2SO_4, and mevalonolactone was recovered from the 40–90-ml fraction. The fractions containing mevalonolactone were concentrated, and aliquots were taken for determination of MVA by the hydroxamate method of Lynen and Grassl (1958). Radioactivity was determined by liquid scintillation counting in a Packard spectrometer. Using this assay, the enzyme exhibited activities that were linear with time over a 2-hr period and linear with enzyme concentration. It was, of course, absolutely dependent on TPNH and HMGCoA.

Purification of HMGCoA Reductase. Sprague–Dawley female rats weighing 150–200 g and fed Laboratory Chow *ad libitum* were decapitated and their livers were quickly removed. All subsequent operations were carried out in cold rooms and refrigerated centrifuges. Two volumes of EDTA buffer (pH 6; 0.03 M EDTA–0.07 M KCl–1 mM dithiothreitol) was added to the liver, which was homogenized in a glass homogenizer with a tightly fitting Teflon pestle for ten passes, or more generally by homogenization in a Waring blender at full speed for 60 sec. The homogenate was centrifuged at 10,000*g* for 15 min, the supernatant solution was centrifuged at 75,000*g* for 90 min. The microsomal precipitate was washed with the same buffer and recentrifuged. With this procedure 40–50 rats provided 80–90 g (wet weight) of microsomal fraction which was suspended in 0.5 M potassium phosphate buffer (pH 6.9) containing 50 mM EDTA and 5 mM mercaptoethanol to a final volume of 340 ml. This suspension contained about 15 mg of protein/ml, 34 ml of 2.75% sodium deoxycholate was added to the microsomal suspension, and the mixture was centrifuged immediately at 75,000*g* for 120 min. The clear supernatant fluid (about 200 ml) was treated with aqueous ammonium sulfate (pH 6.9; saturated at 4°) containing 10 mM EDTA, and the precipitate obtained between 25 and 35% saturation was dissolved in 40–50 ml of 0.01 M potassium phosphate buffer (pH 6.9) containing 1 mM EDTA and 5 mM mercaptoethanol. The solution was dialyzed overnight against 1 l. of the same buffer, which was changed twice. The dialyzed enzyme was then applied to an 8-g column of DEAE-cellulose (2.2 × 20 cm). The column was washed with ten bed volumes of 0.01 M potassium phosphate buffer (pH 6.9) containing 1 mM EDTA and 5 mM mercaptoethanol, followed by two bed volumes of 0.03 M potassium phosphate buffer (pH 6.9) containing the same concentrations of EDTA and mercaptoethanol. The enzyme appeared in the fractions eluted by the 0.03 M potassium phosphate buffer. These fractions were applied to a 1.8 × 12 cm hydroxylapatite column and chromatographed by the sequential addition of 30 ml each of 0.05, 0.10, 0.12, 0.14, 0.16, and 0.20 M potassium phosphate buffer (pH 6.9) containing EDTA and mercaptoethanol.

The reductase generally appeared in the 0.12 M fraction. This fraction contained several other proteins as contaminants, including a yellow protein. However, this preparation was pure enough for most experiments on the properties of the enzyme. The protein contaminants in this fraction were removed almost entirely by Sephadex G-200 chromatography

[2] Following the normal precycling procedure, the DEAE-cellulose was suspended in ethanol, filtered, then resuspended in ether filtered, dried, and stored.

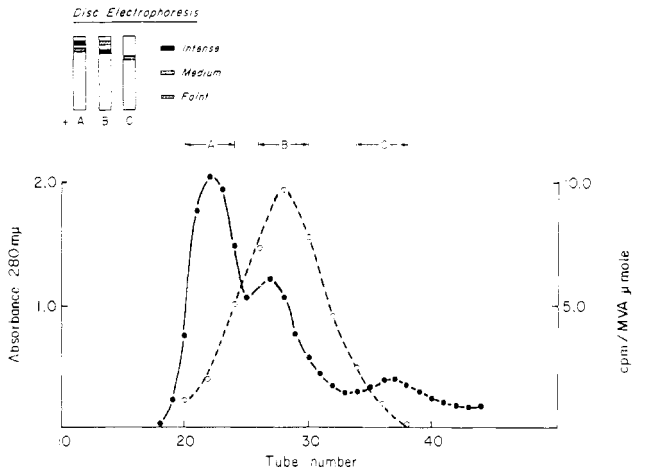

FIGURE 1: Column chromatography of HMGCoA reductase on Sephadex G-200. The column (52 × 0.6 cm void volume, 15 ml) was charged with 14.5 mg of protein and eluted with 0.01 M potassium phosphate buffer containing 1 mM EDTA and 1 mM mercaptoethanol. Each tube contained 0.8 ml of eluate. Fractions A, B, and C were pooled and 30 μg of protein subjected to disc gel electrophoresis. Other details are described in the Methods section. (●——●) Absorbance at 280 mμ; (O------O) HMGCoA reductase activity expressed as counts per minute per micromole of mevalonate.

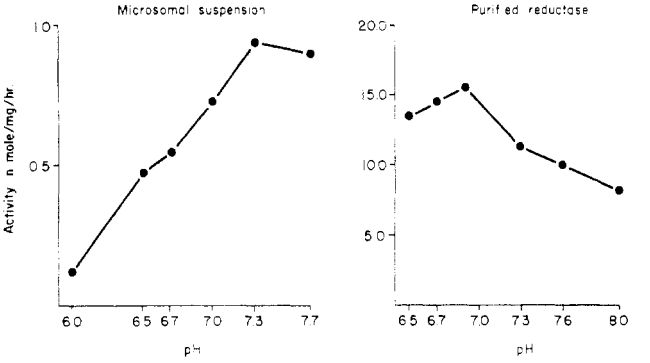

FIGURE 2: Optimum pH of HMGCoA reductase. The reaction mixture contained 10 μmoles of TPN, 4 μmoles of glucose 6-phosphate, 0.7 unit of glucose 6-phosphate dehydrogenase, 3.5 μmoles of dithiothreitol, 300 nmoles (150 nCi) of [3-^{14}C]HMGCoA, 100 μmoles of EDTA, 10 μmoles of MVA, 1 mmole of potassium phosphate buffer at stated pH, and enzyme in a final volume of 5.0 ml. The microsome suspension and purified enzyme contained 4.62 and 1.62 mg of protein, respectively.

in the following manner: The protein was concentrated by dialysis and ultrafiltration in collodion bags (Schleicher & Schuell) to a volume of 1.0 ml and applied to a Sephadex G-200 column (52 × 0.6 cm) equilibrated with 0.01 M potassium phosphate buffer (pH 6.9) containing EDTA and mercaptoethanol as previously outlined. The column was developed with the same buffer, the reductase was only slightly retained being preceded by the yellow protein contaminant. This purified enzyme fraction was used for the molecular weight determination. Disc electrophoresis was carried out on 30-μg samples in a Canalco electrophoresis apparatus with Tris–glycine buffer (pH 8.4) at 4 mA/tube of 5–6% polyacrylamide gel for 30 min.

TABLE I: Purification of HMGCoA Reductase.

Enzyme	Total Protein (mg)	Sp Act. (nmole/mg hr)	Total Act. (nmole/ hr)	Recov (%)
Microsome suspension	6250	0.557	3490	100
Deoxycholate extract	2400	3.50	8400	240
Ammonium sulfate precipitate	548	6.64	3640	104
DEAE-cellulose eluate	30.6	48.0	1468	42
Hydroxylapatite eluate	2.82	180.0	505	14

Results

Purification of HMGCoA Reductase. The results of a typical purification are shown in Table I. The livers of 48 rats were used and the enrichment obtained was 320-fold. The final specific activity of the reductase was 180 nmoles/mg of protein per hr. In some cases activities as high as 600 nmoles/mg of protein per hr were obtained. The enzyme in the crude microsomal form could be stored at −10° with about 10–20% loss of activity per week. The activity observed in the crude extracts, although lower, was of the same order of magnitude as that observed by Guder *et al.* (1968).

Various procedures were tried to obtain solubilization of the enzyme from the crude microsomal preparation. Among the agents used were Triton-X, Tween 20, Tween 80, Triton-X plus sonication, steapsin, crystalline trypsin, acetone treatment according to Linn (1967), deoxycholate and digitonin treatment, and finally sodium deoxycholate alone. Of these, treatment with sodium deoxycholate to provide a final concentration of 0.250% was the only procedure which provided a soluble enzyme preparation. The deoxycholate extracts were treated with ammonium sulfate and the enzyme was obtained between 20–35% saturation. Reductase activity increased at least 2.4-fold after solubilization with deoxycholate. This phenomenon has been noted by Linn (1967) in the case of solubilization with acetone.

After chromatography with hydroxylapatite, the fraction obtained by elution with 0.12 M phosphate buffer contained 70% of the added activity, and was contaminated with a yellow protein and two to four other proteins. The reductase from this fraction was concentrated by ultrafiltration and applied to a Sephadex G-200 column which finally separated the major part of the yellow protein from the reductase activity. The enzyme protein thus obtained was almost entirely free of contaminants, except for a trace of the yellow protein when analyzed by disc electrophoresis (Figure 1).

Molecular Weight Determination. Upon centrifugation of the Sephadex G-200 fraction in a Spinco Model E ultracentrifuge, the reductase had an $s_{20,w}$ value of 9.2–9.5 S. The Stokes radius was determined from chromatography on

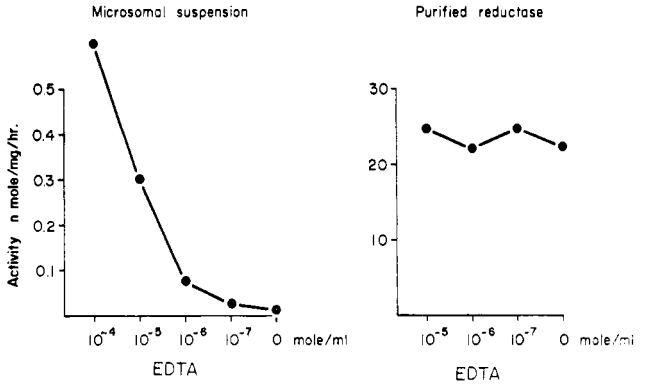

FIGURE 3: Effect of EDTA on the reductase activity. The assay was described in Figure 2. The TPNH-generating system was replaced by 3 μmoles of TPNH. The microsome suspension and the purified reductase contained 5.85 and 2.85 mg of protein, respectively. The enzymes were dialyzed overnight against 0.01 M potassium phosphate buffer containing 5 mM mercaptoethanol.

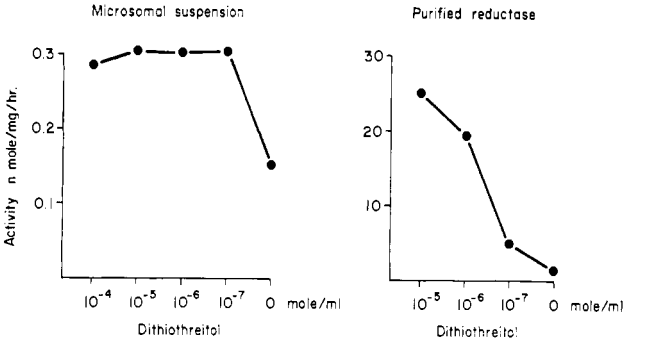

FIGURE 4: Effect of concentration of dithiothreitol on the reductase activity. The assay was described in Figure 2. The enzyme was dialyzed overnight against 0.01 M potassium phosphate buffer containing 1 mM EDTA. The microsome suspension and the purified reductase contained 5.85 and 2.85 mg of protein, respectively.

Sephadex G-200 by the method of Ackers (1964); 14.5 mg of enzyme was placed on a Sephadex G-200 column (52 × 0.6 cm) and the standards used were Blue Dextran and γ-globulin. The Stokes radius was found to be 5.15 mμ. Using the classical equation: $M = 6\pi\eta Nas/(1 - \bar{v}\rho)$ (M is molecular weight; a, the Stokes radius; s, the sedimentation coefficient; $\bar{v}$, the partial specific volume (0.75); η, the viscosity of the medium (0.0101 P); ρ, the density of the medium (0.9983); and N, Avodagro's number) the molecular weight was calculated to be 217,000–226,000.

Properties of the HMGCoA Reductase. As shown in Figure 2, the pH profile of the microsomal suspension showed an optimum between pH 7.3 and 7.7, while the purified reductase had a pH optimum of 7. A difference was also noted between crude and purified reductase with respect to EDTA. As shown in Figure 3, 10 μM EDTA stimulated the activity of the washed microsome suspension, but had no effect on the purified enzyme. The apparent Michaelis constant was determined for each substrate at saturating levels of the other substrate. The observed K_m for HMGCoA was 1.2×10^{-5} M and for TPNH, 8.7×10^{-5} M. Since only one enantiomer of the racemic HMGCoA was reduced, the calculated K_m for HMGCoA becomes 6×10^{-6} M. The plots were linear and showed no curvature.

A thiol requirement for enzyme activity has been previously observed with regard to this enzyme (Durr and Rudney, 1960; Knappe *et al.*, 1959; Siperstein and Fagan, 1966; Kirtley and Rudney, 1967). The activity of the microsomal suspension was doubled by the addition of 100 μM dithiothreitol. On the other hand, a much higher concentration of 10 mM was required to maximally activate the purified reductase 20-fold, suggesting that there may be endogenous thiol in the microsomal system which partially activates the enzyme. These effects are shown in Figure 4. The addition of 10 mg of bovine plasma albumin to the assay reaction mixture stimulated activity by approximately 20%.

As expected in view of the stimulation by sulfhydryl groups, the reductase activity was extremely sensitive to inhibition by low concentrations of sulfhydryl reagents. For example, 10 μM *p*-mercuribenzoate inhibited the reduction 95%.

Effect of Sodium Deoxycholate, Digitonin, and Cholesterol. Sodium deoxycholate is an inhibitor of the HMGCoA reductase both in microsomal suspension and in the purified preparation. However, the enzyme is much more sensitive in the purified form as shown in Figure 5. For example, 50% inhibition of the purified enzyme occurred with 0.025% sodium deoxycholate while the same inhibition is obtained with 0.1% sodium deoxycholate in the crude system. Two percent digitonin caused 80% inhibition. Cholesterol, dissolved in propylene glycol, when added in final concentrations ranging from 10 to 100 μM caused no observable inhibition.

Effect of Acyl-CoA Esters and Other Metabolites. As shown in Table II, the highly purified enzyme was partially inhibited by CoASH, acetyl-CoA, and acetoacetyl-CoA when these compounds were preincubated with the reductase. There was no significant effect on the enzymic activity of another purified preparation when the preincubation step was omitted. This behavior is somewhat analogous to that observed with the yeast enzyme, although the inhibitions of the latter were much greater than we obtained with the rat enzyme. Ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) had no effect on enzymatic activity or stability. Furthermore, the addition of mevalonic lactone to the reaction mixture in concentrations up to 15 μM gave no inhibition.

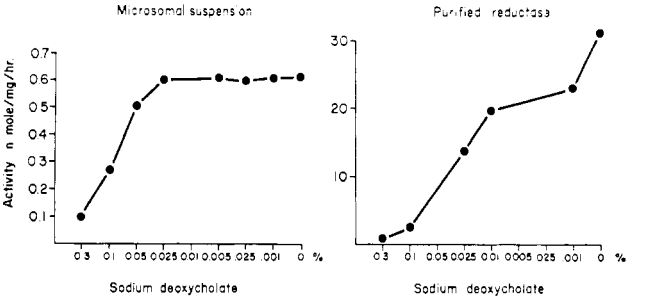

FIGURE 5: Effect of concentration of sodium deoxycholate. The assay was described in Figure 2. The microsome suspension and the purified reductase contained 5.85 and 3.06 mg of protein, respectively.

TABLE II: Effects of Acyl-CoA Esters and CoASH on HMG-CoA Reductase.

Additions (M)	Without Preincubation[a] Act.	Without Preincubation[a] % of Control	With Preincubation[b] Act.	With Preincubation[b] % of Control
None	29.3	100	180	100
Acetyl-CoA				
2.0×10^{-4}	33.3	113	111	62
1.0×10^{-4}	31.5	107	170	93
2×10^{-5}	31.5	107	176	98
Acetoacetyl-CoA				
2.0×10^{-4}	26.9	91	111	62
1.0×10^{-4}	28.2	96	145	81
2.0×10^{-5}	30.2	103	176	98
Coenzyme A				
2.0×10^{-4}	25.9	88	116	64
1.0×10^{-4}	28.9	98	148	82
2.0×10^{-5}	31.1	107	176	98

[a] With preincubation conditions: the reductase was preincubated with 3.5 μmoles of dithiothreitol, 100 μmoles of EDTA, 10 μmoles of MVA, and 1 mmole of potassium phosphate buffer and the acyl-CoA moiety for 10 min at 37°. The reaction was then started by the addition of 3 μmoles of TPNH and 300 mμmoles of [3-^{14}C]HMGCoA. The final volume was 5.0 ml. [b] Without preincubation: all additions were as in *a* except that the 10-min period prior to addition of HMGCoA and TPNH was eliminated. Activity is expressed as nanomoles per milligram of protein per hour. The enzymes used in the with and without preincubation experiments were separate preparations.

TABLE III: Effect of Metal Ions and *o*-Phenanthroline on HMGCoA Reductase.[a]

Additions (M)	Act.	% of Control
None	612.0	100
$FeSO_4 \cdot 7H_2O$		
2×10^{-4}	328.0	54
2×10^{-5}	385.0	63
$FeCl_3 \cdot 6H_2O$		
2×10^{-4}	402.0	66
2×10^{-5}	407.9	67
$MgCl_2 \cdot 4H_2O$		
2×10^{-4}	583.0	95
2×10^{-5}	565.1	92
$ZnSO_4 \cdot 7H_2O$		
2×10^{-4}	628.0	103
2×10^{-5}	603.0	99
None	31.60	100
o-Phenanthroline		
1×10^{-3}	11.34	36
5×10^{-4}	19.74	63
1×10^{-4}	27.60	87

[a] Activity is expressed as nanomoles per milligram of protein per hour. Assay conditions were as described in the legend to Figure 2. The enzyme was dialyzed against 0.01 M potassium phosphate buffer (pH 6.9) containing 5 mM dithiothreitol for 16 hr.

Effect of Metals and o-Phenanthroline. *o*-Phenanthroline is an inhibitor of the yeast enzyme (Kirtley and Rudney, 1967) as well as the purified rat liver reductase (Table III). Some metal ions were also inhibitory, *e.g.*, ferric and ferrous ions, although the ferric ions may have been reduced to ferrous by thiol present. Zinc and magnesium at concentrations of 200 μM had no effect on the activity of the enzyme.

Discussion

In order to assess the significant regulatory role ascribed to this HMGCoA reductase we have solubilized and purified this enzyme to near homogeneity. The enzyme activity located in the microsomes could be solubilized only by low concentrations of sodium deoxycholate. Acetone powders prepared carefully from rat liver microsomes according to the procedure of Linn had, under our conditions, only 20% of the activity of the original microsomes. Extraction of the acetone powders with potassium phosphate buffer containing EDTA and potassium chloride, according to the procedure of Linn, was not successful in our hands, nor are we able to account for this difference.

The subsequent steps after solubilization of the enzyme, *i.e.*, adsorption and elution of the activity from DEAE-cellulose, hydroxylapatite, and Sephadex G-200, were readily reproducible. The enzyme enrichment obtained was about 320-fold. The only other purified preparation of this enzyme reported thus far was that by Kirtley and Rudney from yeast, where the enrichment was 200-fold. A comparison of the properties of these enzymes from both sources, *i.e.*, in rat liver, and yeast, showed similarities with respect to K_m, partial inhibition by acyl-CoA derivatives, CoA and *o*-phenanthroline, and approximate molecular weights. A major difference was observed with respect to inhibition by Fe^{2+} and Fe^{3+}, the yeast enzyme being unaffected by these ions.

It is also of interest that some important parameters of the reductase activity vary considerably in the crude microsomal form and in the solubilized purified form. These were particularly noted in the case of the optimum pH which was shifted and the fact that the purified enzyme became insensitive to EDTA treatment and much more sensitive to sodium deoxycholate and dithiothreitol. As suggested by Linn, the effect of EDTA may be due to the elimination of interference by the HMGCoA cleavage and HMGCoA deacylase enzymes. Fimognari and Rodwell (1965) have shown that deoxycholate is a competitive inhibitor of the reductase from bacterial sources. It is possible that altered properties of the enzyme in the purified form are due to changes in conformational states resulting from protein–protein interactions. Of interest is the differential response of the reductase from microsomal and purified preparations to activation by thiols. The greater concentrations of dithiothreitol required by the purified en-

zyme suggests another possible control parameter for synthesis of mevalonate. Thus the level of available sulfhydryl groups *in vivo* in the microsomal preparation might influence the rate of HMGCoA reduction, *i.e.*, conditions influencing the redox balance of the system with respect to disulfide and thiol groups could thus indirectly regulate mevalonate synthesis. Unfortunately a high degree of purification is required before a reliable spectrophotometric assay becomes feasible. However we did find that the preparation from hydroxylapatite could be measured spectrophotometrically.

With the highly purified preparation of the HMGCoA reductase from rat liver microsomes at hand, we are now able to make a study in depth of the properties of this most interesting enzyme.

References

Ackers, G. K. (1964), *Biochemistry 3*, 723.

Bucher, N. L. R. (1959), *in* Ciba Foundation Symposium, Wolstenholme, G. E., Ed., Boston, Mass., Little, Brown.

Bucher, N. L. R., and McGarrahan, K. (1956), *J. Biol. Chem. 222*, 1.

Bucher, N. L. R., McGarrahan, K., Gould, E., and Loud, A. V. (1959), *J. Biol. Chem. 234*, 262.

Bucher, N. L. R., Overath, P., and Lynen, F. (1960), *Biochim. Biophys. Acta 20*, 491.

Dietschy, J. M., and Siperstein, M. D. (1967), *J. Lipid Res. 8*, 97.

Durr, I. F., and Rudney, H. (1960), *J. Biol. Chem. 235*, 2572.

Fimognari, G. M., and Rodwell, V. N. (1965), *Biochemistry 4*, 2086.

Gould, R. G. (1951), *Am. J. Med. 11*, 209.

Guder, W., Nolte, I., and Wieland, O. (1968), *European J. Biochem. 9*, 273.

Hilz, H., Knappe, J., Ringelmann, E., and Lynen, F. (1958), *Biochem. Z. 329*, 476.

Kirtley, M. E., and Rudney, H. (1967), *Biochemistry 6*, 230.

Knappe, J., Ringelmann, E., and Lynen, F. (1959), *Biochem. Z. 332*, 195.

Langdon, R. G., and Bloch, K. (1953), *J. Biol. Chem. 202*, 77.

Levin, O. (1962), *Methods Enzymol. 5*, 29.

Linn, T. C. (1967), *J. Biol. Chem. 242*, 984.

Lynen, F., and Grassl, M. (1958), *Z. Physiol. Chem. 313*, 291.

Siperstein, M. D. (1965), *in* Symposium on Developmental and Metabolic Control Mechanisms and Neoplasia, Baltimore, Md., Williams & Wilkins, p 428.

Siperstein, M. D., and Fagan, V. M. (1966), *J. Biol. Chem. 241*, 602.

Siperstein, M. D., and Guest, M. J. (1959), *J. Clin. Invest. 38*, 1043.

Tomkins, G. M., Sheppard, H., and Chaikoff, J. L. (1953), *J. Biol. Chem. 201*, 137.

White, L., and Rudney, H. (1970), *Biochemistry* (in press).