the reaction may thus be followed by following the increase in $E_{420}$, which measures the liberation of the dinitrothiophenol. In model reactions the nucleotide was coupled to mercaptoethanol, glutathione and bovine serum albumin.


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**Measurement of Rat Liver Microsomal 3-Hydroxy-3-methylglutaryl-Coenzyme A Reductase Activity, Partially Purified or in Microsomal Suspensions**

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Liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase catalyses the following reaction:

$$3\text{-hydroxy-3-methylglutaryl-CoA} + 2\text{NADPH} + 2\text{H}^+ \rightarrow \text{mevalonate} + 2\text{NADP}^+ + \text{CoASH}$$

Evidence suggests that it is a regulatory enzyme of cholesterol biosynthesis and its activity is generally measured by incubating microsomal suspensions with NADPH and radioactively labelled 3-hydroxy-3-methylglutaryl-CoA, then isolating the radioactive mevalonate formed by t.l.c. (Shapiro *et al.*, 1969), g.l.c. (Hamprecht & Lynen, 1970) or electrophoresis (Berndt & Gaumert, 1971). The method is reliable and sensitive, but slow because of the analytical step needed to separate the radioactive product from the radioactive substrate.

However, for various reasons several other theoretically possible assay methods cannot be used. The rat liver enzyme shows a diurnal variation in activity (Hamprecht *et al.*, 1969; Shapiro & Rodwell, 1971; Edwards & Gould, 1971; Gregory *et al.*, 1972), achieving at the daily maximum a specific activity, when assayed as a microsomal suspension, of about 1.0, and at the daily minimum of about 0.1. Specific enzyme activity is defined as units/mg of protein, where one unit of activity converts 1 nmol of 3-hydroxy-3-methylglutaryl-CoA to mevalonate in 1 min. No sufficiently sensitive chemical or enzymic test for mevalonate is known, so that direct estimation of the mevalonate formed is not practicable. The oxidation of NADPH during the 3-hydroxy-3-methylglutaryl-CoA reductase reaction cannot be used, because microsomal suspensions oxidize NADPH even when no other substrate is added (Gillette *et al.*, 1957; Das *et al.*, 1968). According to Das *et al.* (1968) the initial rate of this NADPH oxidation is in the order of 4 nmol/min per mg of microsomal protein; similar rates have been observed in this laboratory, and there is no known way of selectively inhibiting this 'NADPH oxidase' activity. Assay of rat liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase by measuring the CoASH released does not seem to have been attempted, although the enzyme in pigeon liver microsomal fractions has been assayed in this way by using Ellman's reagent (Ellman, 1959) to estimate the thiol group of CoASH (Hulcher & Oleson, 1973). We have assayed the reductase in rat liver microsomal fraction by using 2-oxoglutarate dehydrogenase (Garland *et al.*, 1965) to measure the CoASH released.

For 3-hydroxy-3-methylglutaryl-CoA reductase assay microsomal fraction was incubated as described previously (Gregory *et al.*, 1972) except that the 3-hydroxy-3-methylglutaryl-CoA was not radioactively labelled. After deproteinization with HCl a sample of the reaction mixture was assayed fluorimetrically for CoASH essentially as
described by Garland et al. (1965). The blank to the reductase incubation contained all components except NADPH. Subtraction of blank from test therefore gives the NADPH-dependent release of CoASH from 3-hydroxy-3-methylglutaryl-CoA catalysed by microsomal fractions. The sensitivity of the assay is determined by the amount of CoASH in the blank compared with that in the test. Experiments showed that the blank contained CoASH from three sources. First, even the best preparations of 3-hydroxy-3-methylglutaryl-CoA contain some CoASH; the contribution from this source can be minimized by using the lowest concentration of 3-hydroxy-3-methylglutaryl-CoA giving saturation of the reductase. Secondly, some chemical hydrolysis of 3-hydroxy-3-methylglutaryl-CoA occurs during incubation and, thirdly, rat liver microsomal fraction has a slight 3-hydroxy-3-methylglutaryl-CoA deacylase activity. The assay method was found to be reliable for reductase activities down to about 0.1 unit/mg of protein; below this, the size of the blank relative to the test makes measurement too inaccurate. Although not as sensitive as the radioactive assay, the assay outlined above is far more rapid and is useful where reductase activity is sufficiently high.

Although methods have been described for partially purifying rat liver microsomal 3-hydroxy-3-methylglutaryl-CoA reductase (Heller & Gould, 1973, 1974; Brown et al., 1973), attempts to assay the partially purified enzyme by a method more convenient than the radioactive one do not seem to have been made. We have found that the enzyme, partially purified to the heat-treated stage by methods slightly modified from those of Brown et al. (1973), contains no appreciable 'NADPH oxidase' activity, so that the reductase activity can be measured as the rate of 3-hydroxy-3-methylglutaryl-CoA-dependent oxidation of NADPH.

Partially purified enzyme was incubated with NADPH and 3-hydroxy-3-methylglutaryl-CoA in a final volume of 0.15 ml and the blank contained all components except 3-hydroxy-3-methylglutaryl-CoA. After incubation, the reaction was stopped, and remaining NADPH destroyed, by acidification with HCl. After neutralization, NADP+ was assayed by adding a portion of the reaction mixture to 1.0 ml of 50mm-Tris-HCl containing 1.5mm-glucose 6-phosphate at pH8.0, then adding glucose 6-phosphate dehydrogenase to rapidly convert all the NADP+ to NADPH. The amount of NADPH formed was measured fluorimetrically. From the stoichiometry of the reductase reaction it is clear that this assay is inherently twice as sensitive as that based on CoASH release.

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