Urinary clearance and metabolism of mevalonate by the isolated perfused rat kidney

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Abstract The urinary excretion and the incorporation into lipids of R[3-^{14}C]mevalonate was investigated in isolated rat kidneys perfused with physiological concentrations of the substrate (80–500 pmol/ml). The clearances of R[3-^{14}C]mevalonate and of the unnatural enantiomer S[5-^{14}C]mevalonate were compared to the glomerular filtration rate measured by the clearance of inulin. Evidence is presented that half of R-mevalonate filtered in the glomerulus is reabsorbed in the tubule whereas S-mevalonate is not reabsorbed. The kidney tubule appears to discriminate between the R and S forms of the mevalonate salt. Urinary excretion and incorporation into lipids accounted for 22% and 46%, respectively, of the uptake of R[3-^{14}C]mevalonate from the perfusate. The label of R[3-^{14}C]mevalonate recovered in lipids was distributed among saponifiable (15%), digitonin-precipitable sterols (18%) and squalene + prenols (67%). Sterol synthesis in the kidney appears to be controlled, at least in part, by the level of circulating R-mevalonate.— **Brunengraber, H., S. B. Weinstock, D. L. Story, and R. R. Kopito.** Urinary clearance and metabolism of mevalonate by the isolated perfused rat kidney. *J. Lipid Res.* 1981. **22:** 916–920.

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R-mevalonate, the product of the rate-limiting reaction of cholesterol biosynthesis was shown in Popják's laboratory to be partially recycled toward acetyl-CoA, acetoacetate, and CO_2 (1, 2). In the rat, the kidney appears to be the primary site of uptake and metabolism, by both sterol and non-sterol pathways, of circulating R-mevalonate (3–6). In a recent report, we showed that R-mevalonate is a normal constituent of human and rat urine (7). The clearance of endogenous R-mevalonate is 29% and 44% of the glomerular filtration rate (GFR) in humans and rats, respectively. To evaluate the contribution of urinary excretion to the overall metabolism of mevalonate, we have investigated the uptake, urinary excretion, and incorporation into lipids of physiological concentrations of R[3-^{14}C]mevalonate in isolated perfused rat kidneys. The clearances of R- and S-mevalonate were compared to the GFR measured by clearance of inulin.

METHODS

Substrates

Commercial RS[5-^{14}C]mevalonate (14.7 μCi/μmol; Schwartz Mann) was purified and resolved by specific phosphorylation of the R enantiomer with mevalonate kinase (EC.2.7.1.36) purified from pig liver to a specific activity of 5 U/mg protein as described by Popják et al. (8). 5-Phospho-R[5-^{14}C]mevalonate was separated from unreacted S[5-^{14}C]mevalonate by chromatography on AG1X8 carbonate resin (Biorad) and hydrolyzed with alkaline phosphatase (9). R[3-^{14}C]mevalonate (51.9 μCi/μmol), RS[5-^{3}H]mevalonate (5 mCi/μmol), [^{3}H(G)]inulin, [^{3}H]- and [^{14}C]toluene were purchased from New England Nuclear. Unlabeled RS-mevalonolactone (Sigma) was hydrolyzed with NaOH and standardized by a coupled spectrophotometric assay using mevalonate kinase, pyruvate kinase, and lactate dehydrogenase (10). All forms of labeled mevalonate were converted to the sodium salt before use.

Kidney perfusions

Adult male and female Sprague-Dawley rats (Charles River) were maintained for 2 weeks on Purina chow made available from 0900 to 1200 each day (11). The animals used were 8–10 weeks old and in a weight range of 250–300 g (males) or 200–250 g (females). Surgery for removal of the right kidney was started around 1300. The technique and apparatus were those of Nishiitsutsuji-Uwo, Ross, and Krebs (12) as modified by Weidemann, Hems, and Krebs (13). Kidneys were perfused with 75 ml of Krebs-Ringer bicarbonate buffer containing 6.5% dialyzed bovine serum albumin (fraction V, fatty acid poor, Miles Biochemicals) and 10 mM glucose. Prior to use, the perfusate was filtered through a 0.45 μm

Abbreviations: GFR, glomerular filtration rate; MVA, mevalonate; TEAC, triethylammonium carbonate; DPS, digitonin-precipitable sterols.

porosity Millipore filter. During the perfusion, the medium was continuously filtered through an on-line 1.2 μm porosity Millipore filter.

After a 15-min equilibration period, R[3-^{14}C]-mevalonate or S[5-^{14}C]mevalonate was added to the perfusate in amounts calculated to achieve initial concentrations ranging from 25 to 800 pmol/ml. At the same time, [^{3}H(G)]inulin was added in amounts equal to five times the activity of [^{14}C]mevalonate. The perfusion was continued for an additional 50 min while urine was collected during alternate 3- and 7-min periods. Perfusate was sampled at the midpoint of each 3-min urine collection. Aliquots of perfusate and urine were incubated for 30 min with acetic acid to eliminate $^{14}CO_2$ and were counted in a liquid scintillation spectrometer. Counts per minute (cpm) were converted to disintegrations per minute (dpm) by recounting the samples after addition of internal standards of [^{3}H]- and [^{14}C]toluene. Inulin and mevalonate clearances were calculated as ml·min^{-1}·g^{-1} of contralateral kidney (14). R-mevalonate was determined by the radioenzymatic assay of Popják et al. (8) using [γ-^{32}P]ATP and mevalonate kinase. Sodium was assayed in perfusate and urine samples by flame photometry (15).

At the end of the perfusion, the kidney was quick-frozen between plates of aluminium tongs precooled in liquid N_2 (16). The material was divided into three aliquots for chloroform–methanol 2:1 (v/v) extraction of lipids (11), perchloric acid-extraction of water-soluble metabolites (17), and determination of the dry weight to wet weight ratio. The incorporation of [^{14}C]mevalonate was measured in the unsaponifiable, digitonin-precipitable sterol (DPS) and saponifiable fractions of the lipid extract (10). Adenine nucleotides were assayed enzymatically in the neutralized perchloric acid extract (17) using a double wavelength Perkin-Elmer 557 spectrophotometer.

Identification of R[3-^{14}C]mevalonate in urine

To 0.05 ml of urine from a kidney perfused with an initial concentration of 500 pmol R[3-^{14}C]mevalonate per ml were added: 1 μCi RS[5-^{3}H]mevalonate, 0.05 μmol unlabeled RS-mevalonate, 1.5 μmol $MgCl_2$, 2.5 μmol ATP, and 0.5 μmol dithiothreitol in a total volume of 0.5 ml of 120 mM potassium phosphate buffer, pH 7.5. The mixture was divided into two equal portions; 0.125 U of mevalonate kinase was added to one of the portions. A second reaction mixture with standards of RS[5-^{3}H]mevalonate and R[3-^{14}C]mevalonate and no urine was processed identically. After a 90-min incubation at 38°C, the reaction mixtures were diluted with 3 ml of 10 mM triethylammonium carbonate (TEAC) buffer, pH 9.7, and loaded onto 0.7 × 10 cm AG1X8 carbonate columns equilibrated with the same buffer (8). The four columns were developed with a common linear gradient made up of 260 ml of 10 mM and 260 ml of 600 mM TEAC buffer. The 3-ml fractions were counted and the cpm was converted to dpm as described above.

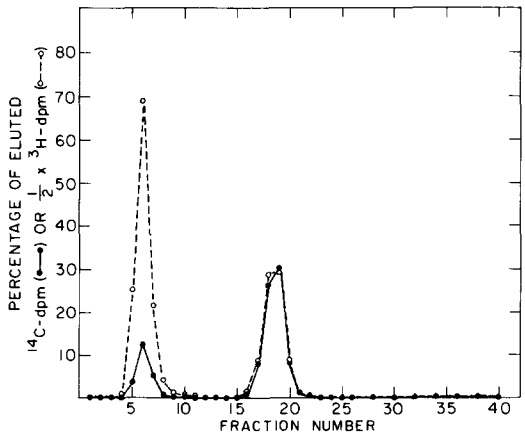

Fig. 1. Chromatogram of a sample of urine from a kidney perfused with R[3-^{14}C]mevalonate after incubation with a standard of RS[5-^{3}H]mevalonate, mevalonate kinase, and Mg-ATP (see Methods). Note that the vertical scale refers to percentage of either total eluted ^{14}C dpm (●) or *half* of total eluted ^{3}H dpm (○). The percentages in all fractions add up to 100% (●) or 200% (○).

RESULTS

The adequacy of the perfused kidney model was assessed by the following parameters. The glomerular filtration rate measured by the clearance of [^{3}H(G)]-inulin was 0.48 ± 0.03 (SEM; n = 27) ml·min^{-1}·g^{-1} at 20 min of perfusion. Subsequent determinations of the GFR at 30 and 40 min were 0.43 ± 0.02 and 0.37 ± 0.02 ml·min^{-1}·g^{-1}, respectively. Sodium reabsorption was 95 ± 0.7% (n = 31) and the [ATP]/[ADP] ratios in the perchloric acid extract of the kidney ranged from 2.0 to 2.7. These parameters are similar to values reported by others (12–14).

The ^{14}C-labeled component in urine from kidneys perfused with physiological concentrations of R[3-^{14}C]mevalonate co-chromatographed with a standard of RS[5-^{3}H]mevalonate before treatment, and with 5-phospho-R[5-^{3}H]mevalonate after treatment with mevalonate kinase and Mg-ATP (**Fig. 1**). When mevalonate kinase was omitted from the reaction mixture, all the ^{3}H- and ^{14}C-label migrated as a single peak eluted at low TEAC concentration (not shown). After reaction with mevalonate kinase, 77% of the

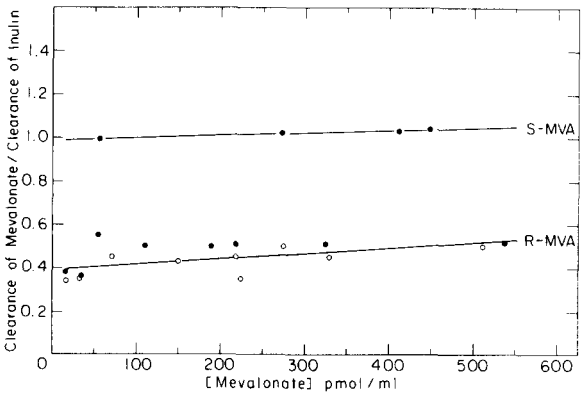

Fig. 2. Ratios of R- and S-mevalonate (MVA) urinary clearances to the clearance of inulin at various substrate concentrations in the perfusate. Kidneys from male (●) and female (○) rats were perfused with [^{3}H(G)]inulin and either R[3-^{14}C] or S[5-^{14}C]-mevalonate. Each point corresponds to one perfusion. Clearances were determined over 3-min periods. The horizontal scale refers to mevalonate concentrations measured at the midpoint of these 3-min periods.

label of the R moeity of RS[5-^{3}H]mevalonate was converted to 5-phospho-R[5-^{3}H]mevalonate which eluted at high ionic strength. An almost equal fraction (76%) of the ^{14}C-label was converted to 5-phospho-R[3-^{14}C]mevalonate which co-migrated with the tritiated derivative. When a mixture of standards of RS[5-^{3}H]mevalonate and R[3-^{14}C]mevalonate was treated and chromatographed in the same manner as the urine sample, 92% of R[3-^{14}C]mevalonate and 94% of the R moiety of RS[5-^{3}H]mevalonate were phosphorylated. We take these observations as evidence that the ^{14}C radioactivity excreted in urine from kidneys perfused with R[3-^{14}C]mevalonate can be entirely ascribed to the parent compound.

In two perfusions, the concentration of R[3-^{14}C]-mevalonate was determined in perfusate and urine by both the radioenzymatic method (8) and by dividing the dpm of ^{14}C/ml by the specific activity of the substrate. Comparison of the two methods by linear regression showed a high correlation (slope = 0.98; r = 0.99). Subsequently the concentration of labeled mevalonate in perfusate and urine was determined by the second method.

The urinary clearances of R- and S-mevalonate are compared in **Fig. 2** to the clearance of [^{3}H(G)]-inulin. The ratio of clearance of R-mevalonate to GFR increased from 0.41 to 0.52 over the physiological range (80–500 pmol/ml) of plasma R-mevalonate concentrations found in the rat (8). The linear regression of the ratio showed a slope of 2.27×10^{-4} ml/pmol, which is significantly different from zero ($P < 0.05$). The ratios measured in perfusions of kidneys from female rats were lower than the ratios from males but the difference between the separate, linear regressions was not significant. The ratio of clearance of S-mevalonate to GFR, measured in perfusions of kidneys from male rats, increased from 0.99 to 1.05 in the same range of S-mevalonate concentration. The slope of the regression was 1.20×10^{-4} ml/pmol and was statistically different from zero ($P < 0.05$).

The uptake of R[3-^{14}C]mevalonate, its excretion in urine, and its incorporation into lipids increased linearly with the concentration of substrate in the perfusate (**Fig. 3**). The values corresponding to female donors were smaller than those of males but the differences were not significant. The regressions were therefore computed on the pooled data from both sexes. The slopes of these regressions were 224, 44.0, and 119 ml·(g dry wt)$^{-1}$·hr^{-1}·mmol^{-1} for the uptake, urinary excretion, and incorporation into lipids, respectively. At a perfusate concentration of 260 pmol/ml, the midpoint of the physiological range of plasma R-mevalonate concentration, urinary excretion and incorporation of R[3-^{14}C]mevalonate into lipids accounted for 22 ± 7 and 46 ± 10% (SD; n = 13) respectively, of the total substrate extracted from the perfusate.

The rate of R[3-^{14}C]mevalonate incorporation into DPS in kidneys from male rats appears to exhibit sigmoidal kinetics in the physiological range of substrate concentration in the rat (**Fig. 4**). It should be noted that the observed data points can be fitted into a linear regression with a slope of 21.7 ml·(g dry wt)$^{-1}$·hr^{-1}·mmol^{-1} and a coefficient of correlation of 0.97. Such a regression would have a wide standard

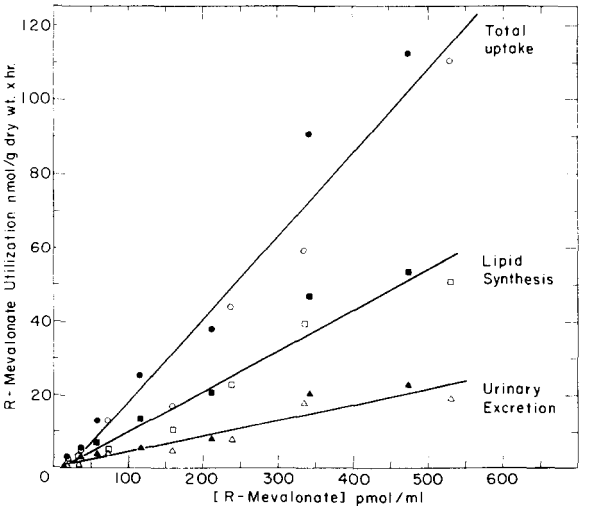

Fig. 3. Cumulative uptake, urinary excretion, and incorporation into total lipids of R[3-^{14}C]mevalonate by perfused kidneys from male (●, ▲, ■) or female (○, △, □) rats. Each point corresponds to one perfusion. The horizontal scale refers to mevalonate concentrations measured at the mid-point of the perfusion with labeled substrate.

deviation of the population (1.14 nmol·(g dry wt)$^{-1}$. hr^{-1}) at the mean R-mevalonate concentration (180 pmol/ml) and an uneven distribution of data points (18). Rates of DPS synthesis in kidneys from female donors were systematically lower than the rates measured in kidneys from male rats. The distribution of R[3-^{14}C]-mevalonate incorporation into lipid classes calculated from linear regressions was as follows: saponifiable fraction (prenoates), 15 ± 5%; DPS, 18 ± 6%; squalene + prenols, 67 ± 12% (SD; n = 13). The incorporation of S[5-^{14}C]mevalonate into the three lipid classes amounted to less than 1% of the incorporation of R[3-^{14}C]mevalonate perfused at similar concentration. This was to be expected as S-mevalonate is not phosphorylated by mevalonate kinase.

DISCUSSION

All the labeled material excreted in urine from rat kidneys perfused with physiological concentrations of R[3-^{14}C]mevalonate was identified as the unmetabolized substrate. In the presence of purified mevalonate kinase and Mg-ATP, this material was phosphorylated to the same extent as the R-moiety of a standard of RS[5-^{3}H]mevalonate. It appears that under the conditions used, the presence of urine in the reaction mixture decreased the degree of phosphorylation of R[3-^{14}C] and R[5-^{3}H]mevalonate equally. The mechanism of this inhibition was not investigated. The specific activity of perfused R[3-^{14}C]mevalonate was not diluted by any detectable unlabeled substrate released by the kidney. The urinary excretion of R-mevalonate is directly proportional to the perfusate concentration and accounts for one-fifth of the total uptake of R-mevalonate by the kidney.

Comparison between the clearances of R- and S-mevalonate and the GFR show that both compounds are filtered like inulin by the kidney glomerulus and that the partial reabsorption process is specific for the R enantiomer. At the level of the proximal tubule, which is the site of reabsorption of carboxylic acids, the pH of the ultrafiltrate is close to plasma pH at which R- and S-mevalonate exist in the salt form (19). It is therefore likely that the proximal tubule discriminates between the salt forms of R- and S-mevalonate.

We have recently reported that the clearance of endogenous R-mevalonate in the rat in vivo is 44% of the GFR (7). The excellent correlation between this figure and the corresponding percentage (46%) reported here suggests that the isolated perfused rat kidney is a suitable model in which to study the urinary excretion of mevalonate. It appears that there is no physiological concentration of circulating R-mevalonate at which the substrate is entirely reabsorbed by the kidney tubule. Thus one can conclude that urinary excretion of R-mevalonate is a physiological process and not simply the consequence of overloading the reabsorption capacity of the kidney (3, 20).

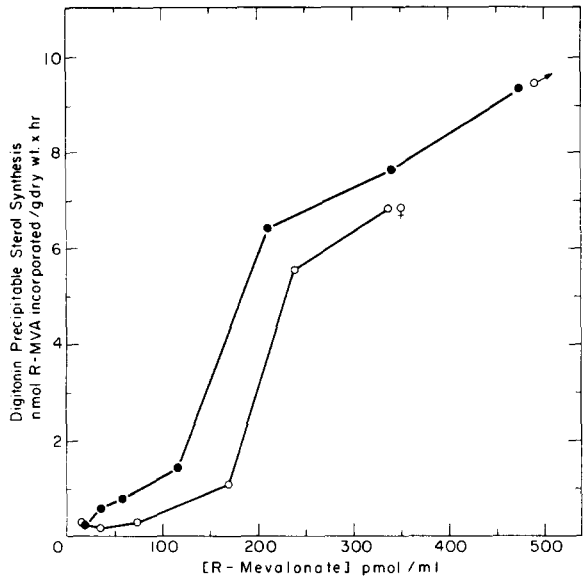

Fig. 4. Incorporation of R[3-^{14}C]mevalonate into DPS by perfused kidneys from male (●) and female (○) rats. Each point corresponds to one perfusion.

Incorporation of R[3-^{14}C]mevalonate into total lipids accounts for 50% of the uptake and 63% of the metabolism of the substrate. The remaining 37% is presumed to represent *i*) mainly $^{14}CO_2$ generated by mevalonate recycling, *ii*) labeled tissue metabolites that are not extractible by chloroform–methanol, and *iii*) R-mevalonate remaining in the vascular and urinary compartments of the kidney. The amount of R-mevalonate assayed radioenzymatically in perchloric acid extracts of the kidneys accounted for less than 2.5% of the substrate taken up during the 50-min experiments.

The apparent sigmoidal character of the incorporation of R[3-^{14}C]mevalonate into DPS (Fig. 4) would indicate that in the physiological range of circulating substrate concentrations, sterol synthesis by the rat kidney is directly dependent upon the supply of R-mevalonate released into the plasma by the liver. Between the extreme values of plasma R-mevalonate concentrations reported by Popják et al. (8), i.e., 80 to 500 pmol/ml, the incorporation of R-mevalonate into DPS increases 9-fold in kidneys from male rats and more than 30-fold in kidneys from female animals.

Andersen and Dietschy (21) have measured sterol synthesis in kidney slices incubated with tritiated water. They report a rate of 17.5 nmol of C2 units/g

wet wt per hr, which is equivalent to 25.4 nmol mevalonate incorporated per gram dry weight per hour. Insofar as they are comparable, our rates of mevalonate incorporation into the DPS of perfused kidney are slightly lower than Andersen and Dietschy's reported values.

Most previous studies on the renal metabolism of labeled mevalonate have been conducted in vivo or in tissue slices using concentrations of substrate two or three orders of magnitude above the physiological range (1–6, 22, 23). In one report, Bardenheier and Popják (9) have incubated rat kidney slices with 1000 pmol R[5-^{14}C]mevalonate per ml of medium. This concentration is twice the highest physiological level for rat plasma (8). Bardenheier and Popják (9) measured a rate of R-mevalonate incorporation into total lipids (saponifiable plus unsaponifiable) of 5.3 pmol per 200 mg kidney slices per 20 min (Fig. 4 of ref. 9). This would correspond to 0.31 nmol/g dry wt per hr. In the isolated rat kidney, perfused with 500 pmol R[3-^{14}C]mevalonate per ml medium, we measured a rate of incorporation into total lipids of 51 nmol/g dry wt per hr (Fig. 3). It follows that the metabolic capacity of kidney slices to convert R-mevalonate to lipid products is only a small fraction (1% or less) of the capacity of the intact perfused kidney. We therefore conclude that when in vivo models are not practicable, the isolated perfused kidney is ideally suited for studying in vitro the renal metabolism of mevalonate.

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REFERENCES

1. Edmond, J., and G. Popják. 1974. Transfer of carbon atoms from mevalonate to n-fatty acids. *J. Biol. Chem.* **249:** 66–71.
2. Fogelman, A. M., J. Edmond, and G. Popják. 1975. Metabolism of mevalonate in rats and man not leading to sterols. *J. Biol. Chem.* **250:** 1771–1775.
3. Edmond, J., A. M. Fogelman, and G. Popják. 1976. Mevalonate metabolism: role of kidneys. *Science* **193:** 154–156.
4. Righetti, M., M. H. Wiley, P. A. Murril, and M. D. Siperstein. 1976. The in vitro metabolism of mevalonate by sterol and non-sterol pathways. *J. Biol. Chem.* **251:** 2716–2721.
5. Wiley, M. H., M. M. Howton, and M. D. Siperstein. 1977. The quantitative role of the kidneys in the in vivo metabolism of mevalonate. *J. Biol. Chem.* **252:** 548–554.
6. Wiley, M. H., M. M. Howton, and M. D. Siperstein. 1979. Sex differences in the sterol and nonsterol metabolism of mevalonate. *J. Biol. Chem.* **254:** 837–842.
7. Kopito, R. R., and H. Brunengraber. 1980. R-Mevalonate excretion in human and rat urine. *Proc. Natl. Acad. Sci. USA.* **77:** 5738–5740.
8. Popják, G., G. Boehm, T. S. Parker, J. Edmond, P. A. Edwards, and A. M. Fogelman. 1979. Determination of mevalonate in blood plasma of man and rat. Mevalonate "tolerance" tests in man. *J. Lipid Res.* **20:** 716–728.
9. Bardenheier, J. W., and G. Popják. 1977. Non-sterol metabolism of mevalonate in vitro: artifacts and realities. *Biochem. Biophys. Res. Commun.* **74:** 1023–1030.
10. Popják, G. 1969. Enzymes of sterol biosynthesis in liver and intermediates of sterol biosynthesis. *Methods Enzymol.* **15:** 393–454.
11. Brunengraber, H., M. Boutry, and J. M. Lowenstein. 1973. Fatty acid and 3-β-hydroxysterol synthesis in the perfused rat liver. *J. Biol. Chem.* **248:** 2656–2669.
12. Nishiitsutsuji-Uwo, J. M., B. D. Ross, and H. A. Krebs. 1967. Metabolic activities of the isolated perfused rat kidney. *Biochem. J.* **103:** 852–862.
13. Weidemann, M. J., D. A. Hems, and H. A. Krebs. 1969. Effects of added adenine nucleotides on renal carbohydrate metabolism. *Biochem. J.* **115:** 1–10.
14. Ross, B. D., F. H. Epstein, and A. Leaf. 1973. Sodium reabsorption in the perfused rat kidney. *Am. J. Physiol.* **225:** 1165–1171.
15. Henry, R. J., D. C. Cannon, and J. W. Winkelman. 1974. Clinical Chemistry, Principles and Techniques. Harper and Row, Hagerstown, MD. 641–644.
16. Wollenberger, A., O. Ristau, and G. Schoffa. 1960. Eine einfache Technik der extrem schnellen Abkühlung grosserer Gewebestucke. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **270:** 399–412.
17. Williamson, J. R. and B. E. Corkey. 1969. Assays of intermediates of the citric acid cycle and related compounds by fluorimetric enzyme methods. *Methods Enzymol.* **13:** 434–513.
18. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, IA. 135–171.
19. Wiley, M. H., S. Huling, and M. D. Siperstein. 1979. Conversion of mevalonolactone to its open-chain salt by a serum enzyme. *Biochem. Biophys. Res. Commun.* **88:** 605–612.
20. Edmond, J. 1974. Ketone bodies as precursors of sterol and fatty acids in the developing rat. *J. Biol. Chem.* **249:** 72–80.
21. Andersen, J. M., and J. M. Dietschy. 1979. Absolute rates of cholesterol synthesis in extrahepatic tissues measured with ^{3}H-labeled water and ^{14}C-labeled substrates. *J. Lipid Res.* **20:** 740–752.
22. Hellstrom, K. H., M. D. Siperstein, L. A. Bricker, and L. J. Luby. 1973. Studies on the in vivo metabolism of mevalonate in the normal rat. *J. Clin. Invest.* **52:** 1303–1313.
23. Raskin, P., and M. D. Siperstein. 1974. Mevalonate metabolism by renal tissue in vitro. *J. Lipid Res.* **15:** 20–25.