Metabolism of plasma mevalonate in rats and humans

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Abstract A circadian rhythm in plasma mevalonate was identified in human subjects. This variation, over a 5-fold range, is paralleled by a rhythm in urinary excretion. No such diurnal change in plasma mevalonate was observed in schedule-fed, light-cycled rats, despite the presence of a pronounced rhythm in liver HMG-CoA reductase and sterol synthesis. A linear correlation was found between liver HMG-CoA reductase activity and the rate of hepatic sterol synthesis. Sterol synthesis accounted for 59% of the HMG-CoA reductase activity. A 4-fold increase in plasma mevalonate following bilateral nephrectomy did not feed back on liver HMG-CoA reductase. Turnover rates for circulating R- and S-mevalonate were determined by the kinetics of tritiated tracers. S-Mevalonate exhibited first-order kinetics with a T½ of 19 to 23 min, while R-mevalonate kinetics could be resolved into two phases with half-lives of 9 and 42 min. The renal uptake of circulating mevalonate was measured by the initial rate of increase in plasma mevalonate immediately following bilateral nephrectomy; this was confirmed by determination of the renal arterio-venous difference. This value ranges between 500 and 600 pmol/min for a 250-g rat.—**Kopito, R. R., S. B. Weinstock, L. E. Freed, D. M. Murray, and H. Brunengraber.** Metabolism of plasma mevalonate in rats and humans. *J. Lipid. Res.* 1982. **23:** 577–583.

Supplementary key words cholesterol • HMG-CoA reductase • biological rhythms

Mevalonate[1] was first shown to be a normal constituent of human and rat plasma in 1972 by Hagenfelt and Hellstrom (1). These investigators developed a gas–liquid chromatographic–mass spectrometric assay which required the pooled blood of three rats and yielded only semi-quantitative values for plasma mevalonate in humans (0.01 μg/ml, equivalent to about 75 nM) and in rats (0.02–0.04 μg/ml). The publication by Popják et al. in 1979 (2) of a radioenzymatic assay for mevalonate provided a method that allows the precise determination of picomole amounts of substrate in a fraction of a milliliter of plasma ultrafiltrate. Using this assay, we identified mevalonate as a normal constituent of human and rat urine, and characterized the urinary excretion process of both R- and S-mevalonate (3, 4)[2].

Hellstrom et al. (6) have suggested that the kidney takes up the bulk of plasma mevalonate and converts it to sterol. Edmond, Fogelman, and Popják (7) have shown that the kidney is the major site of mevalonate recycling in the hypothesized *trans*-β-methyl glutaconate shunt (8–10). This observation has been confirmed by Wiley, Howton, and Siperstein (11).

We present here the results of a series of in vivo studies in humans and rats aimed at elucidating the kinetics and the fate of circulating mevalonate, with emphasis on the roles of the liver and kidney in these processes.

METHODS

Substrates

RS[5-^{3}H]mevalonate (5.7 Ci/mmol), R[3-^{14}C]-mevalonate (51.6 μCi/μmol), RS-3-hydroxy-3-methyl [3-^{14}C]glutaryl-CoA (58.6 μCi/μmol), and ^{3}H- and ^{14}C-toluene counting standards were purchased from New England Nuclear. ^3H_2O (5 Ci/g) was obtained from Amersham. RS[5-^{3}H]mevalonate was purified and resolved by specific phosphorylation of the R-enantiomer with mevalonate kinase as described by Popják et al. (2). 5-Phospho-R[3-^{14}C]mevalonate was prepared by phosphorylation of R[3-^{14}C]mevalonate (2).

Abbreviations: DPS, digitonin-precipitable sterols; HMG-CoA, β-hydroxy-β-methylglutaryl-coenzyme A; MVA, mevalonate; T½, biological half-life.

[1] The physiological enantiomer R-mevalonate is referred to simply as mevalonate. The unnatural enantiomer is designated as S-mevalonate.

[2] Since our first report, we came across an earlier paper by Dimitrieva, Petrunkina, and Khomulo (5) who claimed to have quantitated the urinary excretion of mevalonate in humans. The only identification criteria reported by these authors was that the substance extracted from urine comigrated with mevalonate on paper chromatography. The reported rate of excretion was 30 to 80 mg (200 to 540 μmol) mevalonic acid per 24 hr. This figure is two orders of magnitude higher than the daily secretion rate determined radioenzymatically by us in this and in a previous report (3). We therefore conclude that the material extracted from urine by Dimitrieva et al. cannot be identified as mevalonate.

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Plasma R-mevalonate profile in humans

Three normocholesterolemic volunteers (two men aged 40 and 25; one woman aged 21) were put on a feeding schedule of one meal per day taken at 7 PM. The subjects drank water at regular intervals in order to maintain urine output at about 1 ml/min. After 2 weeks of equilibration, 5-ml blood samples were taken by venipuncture into heparinized tubes every 4 hr. Urine samples were also collected at 4-hr intervals. During the first 2 days of the study, the subjects took their meal (boiled rice, lean roast beef, peas, one pear), but on the third day the meal was omitted. Plasma and urine samples were assayed for R-mevalonate (2, 3) and creatinine (12).

Plasma mevalonate profile in rats

Male Sprague-Dawley rats were placed on a feeding schedule of one meal per day of Charles River chow made available from 9 AM to 12 noon. Water was supplied ad libitum. After 3 weeks of equilibration, the animals, weighing 235–265 g, were divided into nine groups of five or six. Each group was injected intravenously with 30 mCi of ^3H_2O in 0.3 ml of saline at a different point in the same 24-hr cycle. One hr after the injection, the rats were decapitated, blood was collected, and the liver was quickly removed. Serum was used for determination of mevalonate (2) and of the specific activity of water (13). The liver was divided into three aliquots for chloroform–methanol extraction of lipids (13), isolation of microsomes (14), and determination of the dry weight to wet weight ratio. The incorporation of ^3H_2O was measured in the fatty acid and digitonin-precipitable sterol (DPS) fractions (13). The sterols were recrystallized from their digitonides (15) as recommended by Andersen and Dietschy (16). The microsomal suspensions were frozen and kept at −80°C before determination of β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) reductase by the method of Brown et al. (14) as modified by Avigan, Bhatena, and Schreiner (17). Each assay was conducted with three time points at 10, 20, and 30 min. The activities measured were linear with time and with protein concentration.

Turnover rate of R-mevalonate in vivo

Anesthetized rats were injected with 6.5 μCi (1.14 nmol) of R[5-^{3}H] or S[5-^{3}H]mevalonate through a jugular vein catheter. Blood was withdrawn from a femoral artery PE-50 catheter at 3–5 min intervals. Aliquots of plasma were transferred into glass liquid scintillation counting vials containing 1 ml of methanol. After evaporation under a stream of air, a second ml of methanol was added and evaporated in order to eliminate tritiated water derived from R[5-^{3}H]mevalonate metabolism. The residue was dissolved in 0.5 ml of water followed by 15 ml of ACS (Amersham) and counted in a liquid scintillation spectrometer. Duplicate aliquots of plasma were counted without prior evaporation.

Studies in nephrectomized rats

Schedule-fed rats were bilaterally nephrectomized under light ether anesthesia at midnight. They were fed as usual at 9 AM; the amount of food ingested by each rat between 9 and 11 AM was recorded. At 11 AM, the animals were injected intravenously with 0.3 ml of saline containing 30 mCi ^3H_2O. At 12 noon, the rats were decapitated. At midnight on the following day, a second group of rats was sham-operated. At 9 AM, each rat was given the same amount of chow as a weight-matched nephrectomized animal had eaten on the previous day. The sham-operated rats were injected with ^3H_2O at 11 AM and treated in the same way as the nephrectomized rats.

The rate of DPS synthesis and the activity of HMG-CoA reductase were determined in the liver as described above. Mevalonate was assayed in ultrafiltered serum (2).

Another group of rats was nephrectomized and bled from the aorta, at 0, 4, 6, 9 and 12 min after completing surgery. Mevalonate was assayed in the serum.

Kidney catheterization

Male Sprague-Dawley rats weighing 317 ± 6 g (SE, n = 6) were anesthetized with sodium pentobarbital dissolved in water. After performing a midline laparotomy, a 23-gauge needle attached to a Silastic catheter was inserted into the left renal vein past the confluent of the adrenal and spermatic veins. The abdominal aorta was similarly catheterized. The animals were injected with sodium heparin (160 U/kg). After a 10-min equilibration period, 1 ml of blood was withdrawn simultaneously through each of the catheters, by means of a syringe pump, at a rate of 0.5 ml/min. Mevalonate assays were performed in triplicate on ultrafiltered plasma (2).

RESULTS

Fig. 1 shows the profile of plasma concentration and urinary excretion of mevalonate in a 40-year-old normocholesterolemic male subject (HB) equilibrated on a single meal per day feeding schedule. Plasma mevalonate varied sharply over a 5-fold range with a maximum at 7 AM and a minimum at 10 PM. Urinary excretion of mevalonate varied in parallel with plasma concentration. When the daily meal was omitted, no peak was observed in plasma concentration and urinary excretion of mevalonate; both parameters remained at basal levels. During the first two days of the study, the average ratio of

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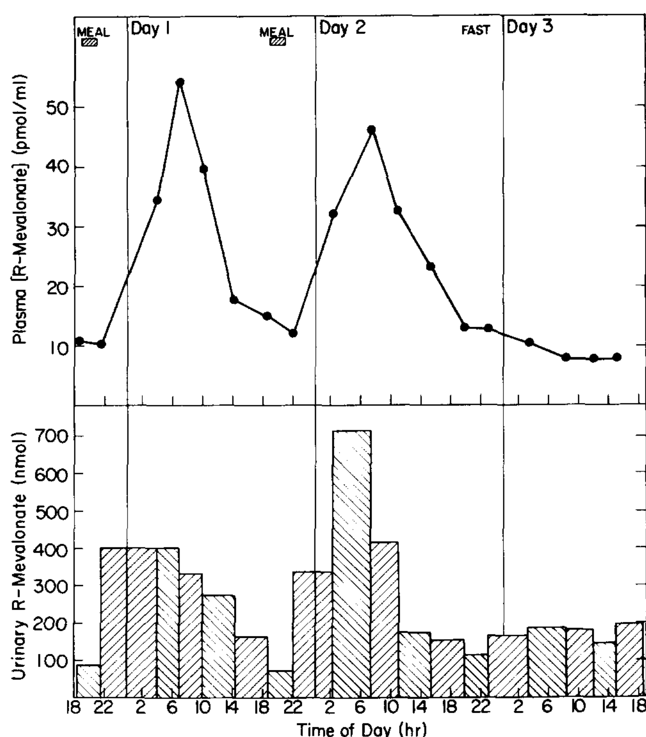

Fig. 1. Nyctohemeral rhythm of plasma mevalonate and of urinary mevalonate excretion in a male human subject equilibrated on a one meal per day feeding schedule. On day 2, the meal was omitted. The 24-hr urinary excretion of mevalonate amounted to 2.06, 1.55, and 0.85 μmol on days 1, 2, and 3, respectively.

mevalonate clearance to creatinine clearance was 0.29 ± 0.02 (SE; n = 13) which is in agreement with our previously published figure (3). In contrast, the ratio of clearances increased significantly ($P < 0.01$) during the fasting period (day 3) to 0.54 ± 0.06 (n = 4). The clearance of creatinine did not vary significantly throughout the study (143 ± 7 ml/min (n = 13) during days 1 and 2 vs. 138 ± 9 ml/min (n = 4) during day 3). The data from the other two human subjects were similar to those shown on Fig. 1: the baseline and the peaks of plasma mevalonate were 10 to 13 nM and 33 to 50 nM, respectively.

Fig. 2 shows the nyctohemeral profile in meal-fed rats of plasma mevalonate, liver HMG-CoA reductase, liver fatty acid, and DPS synthesis. The activity of hepatic HMG-CoA reductase exhibits the well-documented (18) periodicity over a 4-fold range, with a peak occurring 4 hr after the beginning of the meal. The variations in the activity of HMG-CoA reductase parallel the rates of fatty acid and DPS synthesis. In contrast, the meal-induced increase in serum mevalonate concentration, while comparable to the corresponding increase observed in humans, did not reach the level of significance.

Studies in nephrectomized rats

In a previous paper, we showed that within 2 hr after bilateral nephrectomy, the concentration of mevalonate in rat serum increased 4- to 5-fold (3). In the present study, we have investigated the influence of the increase in plasma mevalonate on liver HMG-CoA reductase and DPS synthesis. The data of **Table 1** confirm the 4-fold increase in serum mevalonate in nephrectomized rats over pair-fed sham-operated controls. In contrast, there was no effect of nephrectomy on the activity of liver

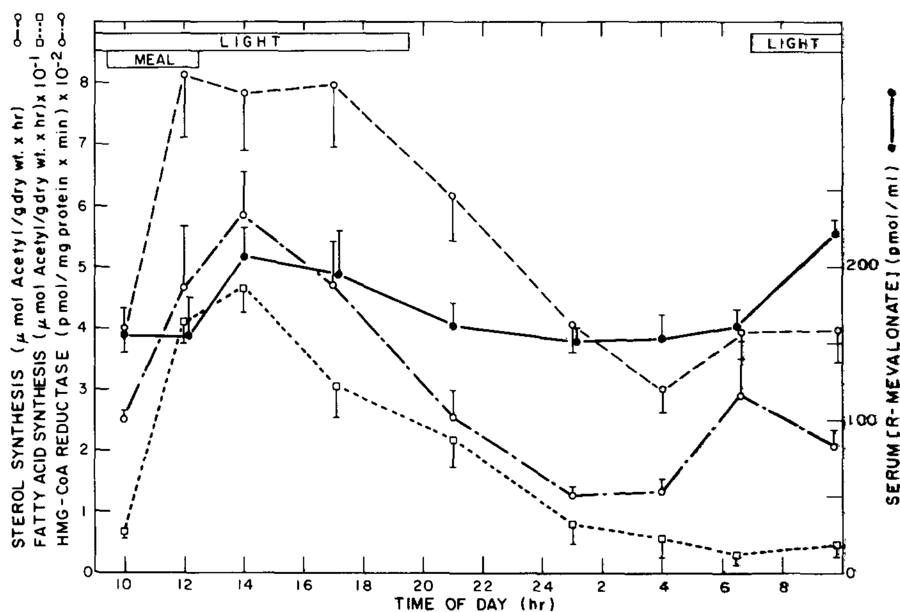

Fig. 2. Nyctohemeral profile of hepatic HMG-CoA reductase (—•—•), sterol synthesis (– – –), and fatty acid synthesis (• • • • •) as well as plasma mevalonate (——) in meal-fed rats. Each point corresponds to five or six animals (mean ± SE).

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TABLE 1. Effect of bilateral nephrectomy on serum mevalonate concentration, on the activity of liver HMG-CoA reductase, and on the rate of sterol synthesis in the liver of meal-fed rats

	Serum Mevalonate	Liver Microsomal HMG-CoA Reductase	Liver Sterol Synthesis
	nM	*pmol/mg protein × min*	*µmol acetyl/g dry wt × hr*[a]
Nephrectomized (7)	642 ± 35	551 ± 51	6.2 ± 0.4
Sham controls (7)	156 ± 13	547 ± 86	8.0 ± 0.8
P	<0.001	ns	ns

[a] 1.31 × µmol $^3H_2$ incorporated/g dry wt × hr (22).
The animals were killed 12 hr after bilateral nephrectomy and 1 hr after injection of 30 mCi ^3H_2O.

HMG-CoA reductase and on the rate of hepatic DPS synthesis.

In a different group of rats, we measured the initial rise in plasma mevalonate after bilateral nephrectomy (**Fig. 3**). After a 5-min lag, the concentration of mevalonate in plasma increased linearly with a slope of 12 nM/min (r = 0.99).

Renal uptake of mevalonate in rats

The uptake of mevalonate by the rat kidney was determined by measuring the arterio-venous difference in mevalonate concentration across the kidney of anesthetized rats. During a single passage through the kidney, the concentration of plasma mevalonate decreased by 53 ± 2% (SE; n = 6) from 122 ± 6 to 56 ± 4 nM. In a similar study, Popják et al. obtained a figure of 34 ± 8% (n = 6; calculated from Table 3 of ref. 2).

Turnover of plasma mevalonate in rats

The turnover of circulating mevalonate was measured by the decrease in the plasma radioactivity of a tracer of R[5-^{3}H]mevalonate injected intravenously. When plotted on semi-logarithmic coordinates (**Fig. 4**), the kinetics of R-mevalonate are best described as a biphasic process. The initial rapid uptake of label lasts for 10 to 15 min and is followed by a slower uptake. The half-life (T½) of these processes was 9.04 ± 0.71 (n = 7) and 41.6 ± 8.9 (n = 4) min, respectively. When the aliquots of plasma were not evaporated before counting (see Methods), the T½ of the slow phase was increased to 94 ± 14 min (n = 4). These T½ are similar to those

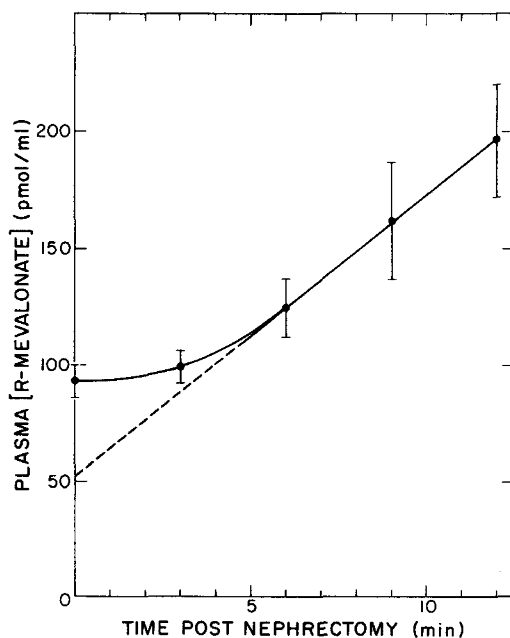

Fig. 3. Early increase in plasma mevalonate in rats following bilateral nephrectomy. Each point corresponds to four animals (mean ± SE). The linear regression was calculated using the 6, 9, and 12-min time points.

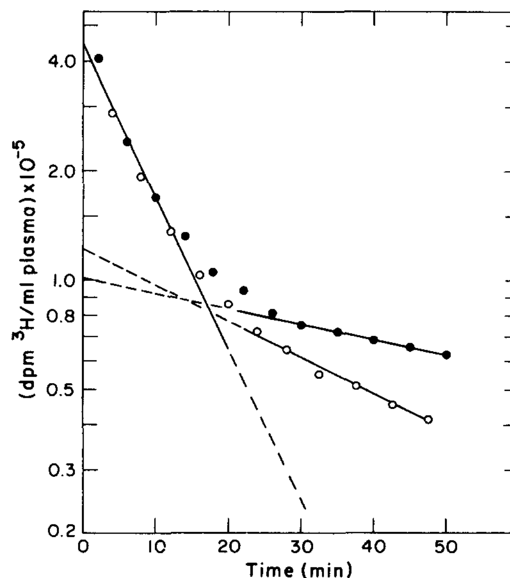

Fig. 4. Kinetics of plasma mevalonate in the rat. A tracer amount of R[5-^{3}H]mevalonate (6.5 µCi, 1.14 nmol) was injected in the jugular vein of a normal rat. Samples of arterial plasma were counted either without processing (●) or after two evaporations to remove ^3H_2O (○).

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observed in humans by Popják et al. (2). The distribution volume of R[5-^{3}H]mevalonate ranged from 26 to 33% of body weight.

The uptake from the plasma of a tracer of S[5-^{3}H]mevalonate followed first-order monophasic kinetics (not shown). T½ was 19 to 23 min and the distribution volume was 29 to 32% of body weight (n = 3). S-mevalonate was used as a control substrate to differentiate the processes of distribution and metabolism.

DISCUSSION

The report by Popják et al. (2) suggested that the level of rat plasma mevalonate varies during the day–night cycle and correlates with the activity of hepatic HMG-CoA reductase. This prompted us to look for diurnal variations in plasma mevalonate in humans and rats. In order to optimize the search for a diurnal rhythm, the human subjects and the rats were pre-equilibrated on a one meal per day feeding schedule. This regimen induces sharp variations in rates of lipogenesis (19) and in activity of HMG-CoA reductase (18) in the rat. Under such conditions, the concentration of mevalonate in human plasma follows a clear oscillatory pattern (Fig. 1). The abolition of the oscillation when the daily meal was omitted suggests that the rhythm is induced by feeding and not by an intrinsic biological clock. During the first 2 days of the study, urinary excretion of mevalonate paralleled plasma concentration. The glomerular filtration rate (measured by the clearance of endogenous creatinine) and the ratio (clearance of mevalonate/clearance of creatinine) remained constant. When the subject fasted (day 3), the fractional excretion of mevalonate increased. The physiological significance of this phenomenon is unclear. We were expecting that, during fasting, the fractional excretion of mevalonate would decrease thereby sparing this biosynthetic substrate. The increase in fractional excretion of mevalonate during fasting may be a mechanism diverting carbon from the sterol synthesis pathway. More likely, this phenomenon may simply reflect an alteration, induced by fasting, in renal tubular reabsorption. As the mevalonate content of the meal was not assayed, we cannot exclude the possibility that dietary mevalonate might contribute to the meal-induced increase in serum concentration. The lateness of the peak in serum mevalonate (12 hr after the meal) argues against this hypothesis. Popják et al. (2) have shown that the peak in plasma mevalonate is reached 15 min after oral ingestion.

In schedule-fed rats, the peak in serum mevalonate occurred 4 hr after the beginning of the meal. The increase in serum mevalonate over the baseline (40 nM) was comparable to that observed in humans. The lack of statistical significance of this increase is ascribed to the higher baseline level of serum mevalonate in rats (150 nM) as compared to humans (10–13 nM).

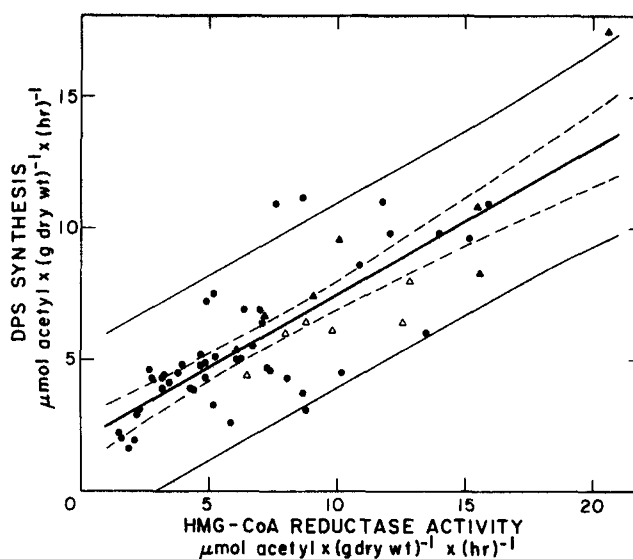

Fig. 5. Correlation between the activity of liver HMG-CoA reductase and the rate of hepatic sterol synthesis in meal-fed rats. Both parameters are expressed as μmol acetyl/g dry wt × hr. Calculations were based on a concentration of microsomal protein of 40 mg/g wet weight of liver. The data from three groups of rats are presented: controls (●; see Fig. 2); nephrectomized (▲; Table 1), and sham-operated (△; Table 1). The limits of confidence of the population (continuous curves) and of the regression (broken curves) were calculated according to Snedecor and Cochran (23).

We have previously reported that the concentration of serum mevalonate in the rat increased about 5-fold in the hours following bilateral nephrectomy (3). We offered two hypotheses to explain the higher steady state in plasma mevalonate. First, the higher serum mevalonate concentration might feed back on the activity of liver HMG-CoA reductase. Alternatively, the rise in serum mevalonate might increase the uptake of the substrate by peripheral tissues until this uptake balances liver production. In the present study, we show that the activity of HMG-CoA reductase and the rate of sterol synthesis in the liver remain unchanged 12 hr after nephrectomy (Table 1). This suggests that the increase in serum mevalonate after nephrectomy increases the uptake of substrate by peripheral tissues until this uptake balances liver production.

The notion that HMG-CoA reductase is the rate-limiting step in cholesterol synthesis (20) implies that there should be a tight correlation between the activity of the enzyme and the rate of sterol synthesis. We have studied this correlation by expressing both parameters in the same units, that is μmol acetyl/g dry wt × hr (**Fig. 5**). We found a linear correlation (r = 0.86) with a slope equal to 0.59. In other words, if the activity of HMG-CoA reductase assayed in vitro reflects the activity pres-

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ent in vivo, 41% of the mevalonate produced in the liver during the 1-hr experiment is not found in DPS. The fates of the missing fraction of mevalonate production are most likely *i*) intermediates of cholesterol synthesis that are not precipitated by digitonin (mostly squalene); *ii*) ubiquinone and dolichol synthesis; *iii*) export of mevalonate into plasma; *iv*) mevalonate recycling in the liver itself (8, 9).

The distribution volumes of both R and S mevalonate appear to be somewhat larger than the extracellular fluid (20% of body weight). This is probably an artifact generated by the very rapid uptake of mevalonate during the first passage of the injected bolus through the kidneys. This initial uptake presumably reduces the amount of mevalonate that distributes in the extracellular fluid. This in turn leads to an underestimation of the extrapolated initial plasma activity and to an overestimation of the distribution volume. It seems reasonable to conclude that the distribution volumes of both R and S mevalonate in the rat correspond to the extracellular fluid.

Hellstrom et al. (6) have previously measured the turnover rate of large doses of RS[5-^{3}H] and R[1-^{14}C]mevalonate in rats. As they observed biphasic kinetics with similar half-lives for both tracers, they implied that the kinetics of R and S mevalonate were identical. Our data show that the T½ of R and S[5-^{3}H]mevalonate are very different (42 min for the slow phase of R[5-^{3}H]mevalonate uptake vs. 21 min for the single phase of S[5-^{3}H]mevalonate uptake). It appears that Hellstrom et al. did not evaporate their plasma samples before counting in order to eliminate ^3H_2O derived from R[5-^{3}H]mevalonate metabolism. This leads to a great overestimation of the T½ of the slow phase of R[5-^{3}H]mevalonate uptake (94 vs. 42 min when our samples were evaporated before counting, Fig. 4). The T½ of RS[5-^{3}H]mevalonate measured by Hellstrom et al. (6) probably represents the average between the very rapid uptake of S[5-^{3}H]mevalonate and the artifactually slow uptake of R[5-^{3}H]mevalonate. This average (28 min) is coincidentally close to the T½ of R[1-^{14}C]mevalonate (24.6 min).

The initial linear rise in plasma mevalonate following bilateral nephrectomy (12 nM/min) would reflect the renal uptake of mevalonate (before compensatory or feedback mechanisms are activated). Assuming that mevalonate is distributed in the extracellular fluid (50 ml in a 250-g rat), the renal uptake would amount to 600 pmol/min. A similar figure was obtained from the direct measurement of the arterio-venous difference in plasma mevalonate across the kidney (66 ± 5 nM; n = 6). As the total renal plasma flow in a 250-g rat is about 8 ml/min (21), the renal uptake of mevalonate amounts to 528 pmol/min. These data confirm that the kidney is the major "sink" for circulating mevalonate. The physiological and biochemical implications of this finding warrant further investigation.

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