The Shunt Pathway of Mevalonate Metabolism in the Isolated Perfused Rat Liver*

Shelley B. Weinstock†, Ron R. Kopito, Gerda Endemann, John F. Tomera, Evelyne Marinier, Donald M. Murray, and Henri Brunengraber‡

From the Department of Nutrition and Food Science, Massachusetts Institute of Technology. Cambridge, Massachusetts 02139

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The shunt pathway of mevalonate metabolism (Edmond, J., and Popják, G. (1974) J. Biol. Chem. 249, 66–71) has been studied in isolated livers from fed rats perfused with physiological concentrations of variously labeled ['4C]mevalonates. The measured rates of 14CO2 production were converted to rates of mitochondrial acetyl-CoA production from mevalonate by methods which take into account underestimate of metabolic rates derived from 14CO2 production. Our data confirm that the shunt pathway leads to mitochondrial acetyl-CoA. The apparent negligible rate of mevalonate shunting in liver, previously reported by others, stems from the very low contribution (≈ 0.1%) of plasma mevalonate to total mevalonate metabolism in the liver. This contribution was assessed from the relative incorporations of 3H2O and [5-14C]mevalonate into sterols. In livers from fed rats, the shunt diverts about 5% of the production of mevalonate. The total rate of mevalonate shunting in the liver is about 200 times greater than in two kidneys. The liver is therefore the main site of mevalonate shunting in the rat.

The shunt pathway of mevalonate metabolism hypothesized by Popják in 1970 (1) is a series of reactions which links the pathways of cholesterol synthesis and leucine catabolism. Branching between the sterol and the shunt pathways occurs at the level of dimethylallyl pyrophosphate which is converted sequentially to dimethylallyl alcohol, β-methylcrotonate, and β-methylcrotonyl-CoA. The latter substrate is an intermediate in the pathway of leucine catabolism to HMG-CoA. The latter substrate is an intermediate in the pathway of cholesterol synthesis and leucine catabolism. The apparent negligible rate of mevalonate shunting was assessed by incorporation of 14C into liver sterols. The rate of mevalonate shunting was assessed by the incorporation of 14C into CO2 and n-fatty acids. Rates of 14CO2 production were converted to rates of acetyl-CoA production from mevalonate using methods that estimate under-recovery of label via exchange processes. Briefly, the extent of 14CO2 reincorporation into compounds nonvolatile in acid was assessed by the recovery of 14CO2 from livers perfused with a tracer of [14C]bicarbonate (18). The yield of label from mitochondrial [14C]acetyl-CoA to 14CO2 was obtained from the differential yield of 14CO2 from livers perfused with tracers of [2-14C]- and [1-14C]-β-ketoisocaproate (4-methyl-2-oxovalerate) (19).

We present evidence that the liver is the main site of the shunt pathway of mevalonate metabolism. The shunt yields mitochondrial HMG-CoA and diverts about 5% of the mevalonate entering the cholesterol synthesis pathway in livers from fed rats.

EXPERIMENTAL PROCEDURES

Materials—Enzymes and coenzymes were purchased from Boehringer and P-L Biochemicals. (+)-Hydroxycitric acid lactone from Garcinia cambogia was hydrolyzed with 3.1 eq of NaOH for 1 h at 80 °C (20). Mevinolin was a generous gift from A. W. Alberts (Merck Sharp and Dohme). Standards of nystatine, palmatate, oleate, linoleate, cholesterol, and desmosterol were purchased from methylcrotonyl-CoA carboxylase.

Evidence supporting this pathway came from Edmond and Popják (2) and Fogelman et al. (3) who injected [2-14C]- or [5-14C]mevalonate into rats and humans. Label was recovered in ketone bodies and in products of ketone body and acetyl-CoA metabolism (CO2, n-fatty acids). Further support for the shunt pathway was reported by Brady et al. (4), based on the distribution of 14C in β-hydroxybutyrate excreted by rats following injection of mevalonate specifically labeled on different carbons.

The bulk of plasma mevalonate (endogenous or exogenous) is taken up by the kidneys (5–7). The three main fates of mevalonate in the kidney are: incorporation into squalene and sterols (3, 5, 8–12), excretion in urine (7, 11–13), and metabolism via the shunt (8–10, 12, 14, 15).

In vivo (2, 10) and in vitro (9) studies using [2-14C]- or [5-14C]mevalonate have suggested that metabolism of mevalonate via the shunt is negligible in the liver. We suspected that the apparent inability of the liver to shunt mevalonate stems from poor permeation of exogenous [14C]mevalonate into the liver cell. Comparison between rates of [14C]mevalonate (5, 9, 10) and of 3H2O (16, 17) incorporation into liver sterols shows that the contribution of extracellular mevalonate to total mevalonate metabolism in the liver is indeed very small. We addressed this problem by perfusing livers with 3H2O and physiological concentrations of variously labeled [14C]mevalonates. The fractional contribution of exogenous mevalonate to total metabolism of mevalonate was assessed by the incorporation of 3H and 14C into liver sterols. The rate of mevalonate shunting was assessed by the incorporation of 14C into CO2 and n-fatty acids. Rates of 14CO2 production were converted to rates of acetyl-CoA production from mevalonate using methods that estimate under-recovery of label via exchange processes. Briefly, the extent of 14CO2 reincorporation into compounds nonvolatile in acid was assessed by the recovery of 14CO2 from livers perfused with a tracer of [14C]bicarbonate (18). The yield of label from mitochondrial [14C]acetyl-CoA to 14CO2 was obtained from the differential yield of 14CO2 from livers perfused with tracers of [2-14C]- and [1-14C]-β-ketoisocaproate (4-methyl-2-oxovalerate) (19).

We present evidence that the liver is the main site of the shunt pathway of mevalonate metabolism. The shunt yields mitochondrial HMG-CoA and diverts about 5% of the mevalonate entering the cholesterol synthesis pathway in livers from fed rats.

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† Present address, Harvard School of Public Health, Boston, MA 02115.
‡ Supported by Established Investigatorship 78230 from the American Heart Association. Present address, Notre Dame Hospital, Metabolic Unit, Montreal, Quebec H2L 4K8, Canada. To whom reprint requests should be addressed.
1 The physiological enantiomer R-mevalonate is referred to simply as mevalonate.
2 The abbreviation used is: HMG-CoA, β-hydroxy-β-methylglutaryl-CoA.
Mevalonate Shunt Pathway in Liver

Supelco. Lanosterol (70-80%) was purchased from Sigma; the major impurity was dihydroxlanosterol. Squalene (>99%) was obtained from Sigma. Farnesiose methyl ester was synthesized as described by Cornforth and Popjak (21).

RS-[1-14C]Mevalonate (7.2 µCi/µmol). R-[2-14C] Mevalonate (63 µCi/µmol). and ACS counting fluid were purchased from Amersham Corp. RS-[4,5-14C] Mevalonate (99.5 µCi/µmol) was obtained from Research Products International Corp. RS-[5-14C] Mevalonate (14.7 µCi/µmol) was supplied by Schwarz/Mann. R-[3-14C] Mevalonate (51.9 µCi/µmol), [4,8,12,13,17-2H] Squalene, [4-14C] cholesterol, [14C]- and [3H]-isotope counting standards and Oxifluor-CO2 scintillation fluid were purchased from New England Nuclear. All samples of labeled RS-mevalonate were purified and resolved by a specific phosphorilation of the R-enantiomer (6, 14) with mevalonate kinase purified from pig liver to a specific activity of 5 units/mg of protein as described by Popjak et al. (6).

Liver Perfusions—Adult male and female Sprague-Dawley rats (Charles River) were maintained for 2 weeks on Purina chow made available from 9 a.m. to 12 noon each day. The animals used were 8-10 weeks old and weighed between 250 and 300 g (males) and 200 and 250 g (females). Surgery for removal of the liver was started around 10 a.m. The surgical technique and the perfusion apparatus were described previously (16). Livers were perfused with 150 ml of Krebs-Ringer bicarbonate buffer containing 4% dIyzed bovine serum albumin (fraction V, fatty acid poor, Miles Biochemicals) and 15 mm glucose.

After a 30-min equilibration period, labeled R-mevalonate was added to the perfusate in amounts calculated to achieve an initial concentration of 250-400 nM, except where indicated. These concentrations are in the physiological range of plasma mevalonate concentration in the rat (80-500 nM, Ref. 6). The perfusion was continued for an additional 90 min throughout which perfusate was sampled every 10 min. Aliquots of perfusate were incubated for 30 min with acetic acid to eliminate 14CO2 and were counted in a liquid scintillation spectrometer. The effluent 14CO2 from the oxygenator and the 14CO2 present in the perfusate bicarbonate pool at 120 min were trapped in Oxifluor, as described previously (22).

Analytical Techniques—the frozen tissue was divided into 2 aliquots; one-third of the dry weight was used to obtain chloroform/methanol (2:1, v/v) extraction of lipids which were analyzed as described previously (12, 16), with the following exception. The fatty acids were methylated with diazomethane and separated by preparative gas chromatography. A 2.4-m stainless steel column (4 mm, inner diameter) was packed with 3% SE-30 on Chromosorb W/HP (80/100 mesh). Temperatures were used Varian Aerograph model 1200 gas liquid chromatograph equipped with a flame ionization detector. The column was operated at 240°C. Injection port and detector were set at 300°C; outlet tube was set at 350°C. Gas carrier was nitrogen (70 ml/min). Retention times of methyl caproate, methyl caprate, and methyl caprate were 65, 45, and 15 min, respectively. Individual methyl esters were trapped in U-tubes (21) filled with silanized glass wool and cooled in liquid N2.

Mevalonate was assayed in ultrafiltrates from the perfusate by the method of Popjak et al. (6), with the following exception. The perfusate was first divided by the 14COP recovery factor. Second, an additional correction was applied to the production of 14CO2 from mevalonate metabolism, carbon 1 of the substrate is rearranged to 14CO2. We have recently shown (18) that this is applicable to the transfer to 14CO2 of label from acetyl-CoA derived from [14C]mevalonate (see Footnote 6 of Table I).

RESULTS

Throughout the 2-h recirculating perfusion, livers released mevalonate linearly (r = 0.92) at a rate of 200 pmol/min or 5.4 ± 0.43 (±S.E.; n = 6) nmol/g, dry weight × h. The rate of accumulation was not affected in experiments where a bolus of 250-400 nM of labeled mevalonate was added to the perfusate at 30 min. Production of endogenous unlabeled mevalonate was used to assess the dilution (less than 45%) of the specific activity of the tracer between 30 and 120 min of the experiment.

The uptake of [14C] mevalonate was calculated from the linear decrease over 90 min of the acid-stable radioactivity in the perfusate. This decrease amounted to about 20% of the initial concentration. That practically all of the acid-stable radioactivity in the final perfusate was [14C]mevalonate was ascertained as follows. First, ultrafiltration of the perfusate did not affect its acid-stable radioactivity. Second, the 14C-labeled component present in the ultrafiltrate co-chromatographed with a standard of R-[5-3H] mevalonate before treatment and with 5-phospho-R-[5-3H] mevalonate after treatment with mevalonate kinase and Mg-ATP (11). Practically equal fractions of the 14C (98%) and 3H (96%) label were converted to the phosphorylated derivative.

Total rates of sterol synthesis were assessed in separate perfusion experiments by the incorporation of 3H2O. There was no significant difference in the rates of sterol synthesis between livers from males (3.28 ± 0.11 µmol of mevalonate equivalent/g, dry weight × h; n = 5) and from females (4.14 ± 1.19; n = 6). Similarly, rates of hepatic sterol synthesis in vivo (16) were not significantly different in males (5.71 ± 0.68; n = 8) and females (6.76 ± 0.94; n = 8).

Experiments with [1-14C] Mevalonate—In the third reaction of mevalonate metabolism, carbon 1 of the substrate is released as CO2 by pyrophosphomevalonate decarboxylase. Since the intracellular accumulation of [1-14C] mevalonate and of its phospho- and pyrophospho-derivatives is negligible, the uptake of [1-14C] mevalonate should be balanced by an equivalent production of 14CO2. We have recently shown (18) that when metabolic production of 14CO2 by the perfused liver is simulated by a constant infusion of NaH14CO3 into the perfusate, the recovery of the infused label in the effluent gas of the oxygenator, plus the pool of bicarbonate in the final perfusate, is considerably less than 100%. In livers from male and female fed rats, the recovery of NaH14CO3 was 63 ± 4% (n = 5) and 73 ± 5% (n = 6), respectively. In livers from male and female rats perfused with 650 nM of [1-14C] mevalonate, the production of 14CO2 amounted to 58 ± 10% (n = 5) and 67 ± 5.6% (n = 11) of the uptake of [1-14C] mevalonate. We therefore concluded that the recovery factors measured in experiments with NaH14CO3 were applicable to the production of 14CO2 from [14C] mevalonate.

If all the metabolism of [1-14C] mevalonate goes through pyrophosphomevalonate decarboxylase, none of the end products of either the sterol or the shunt pathway should be labeled. An alternate series of reactions for the conversion of mevalonate to HMG-CoA was considered (24). In this scheme, mevalonate is first dehydrated to Δ5 or Δ5-enamido mevalonate which is converted to its CoA derivative Oxidation of the primary alcohol function followed by hydration yields HMG-CoA. This series of reaction does not involve the

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G. Popjak, personal communication.
loss of carbon 1 of mevalonate at pyrophosphomevalonate decarboxylation. If the shunt pathway occurred via Δ2- or Δ3-anhydrolevalonate, [1-14C]mevalonate would yield [1-14C]acetyl-CoA, and one would expect to find label in fatty acids and sterols. No detectable labeling of fatty acids or sterols was found in livers perfused with either 0.65 or 40 μM [1-14C]mevalonate. Under our analytical conditions, we could have detected a level of labeling in fatty acids or sterols corresponding to 0.01% of the uptake of [1-14C]mevalonate. Taken as a whole, these experiments with [1-14C]mevalonate and with NaH14CO3 demonstrate that in the liver (i) all the metabolism of mevalonate occurs via pyrophosphomevalonate decarboxylation, and (ii) the shunt pathway of mevalonate metabolism does not proceed via anhydrolevalonate.

Experiments with Other 14C-Mevalonates—Livers from male and female rats were perfused with either 400 nm of [5-14C]mevalonate or 250 nm of [4,5-14C]mevalonate. These tracers yield [1-14C]- or [1,2-14C]acetyl-CoA whose label is expected to be found in CO2 and in n-fatty acids. The uptake of mevalonate and the measured production of 14CO2 were roughly proportional to the concentration of substrate and did not show any significant sex difference (Table I). Production of 14CO2 accounted for about 1% of the uptake of mevalonate. Note that this is a minimal estimate of the rate of the shunt pathway since a sizable fraction of the label of [1-14C]acetyl-CoA is lost from the tricarboxylic acid cycle via exchange reactions with amino acids and glycolytic intermediates (25). In addition, a fraction of 14CO2 generated in the cycle is reincorporated via exchange processes. The measured rates of 14CO2 production were converted to rates of [14C]acetyl-CoA production by methods described previously (18, 19), using the recovery coefficients listed under "Experimental Procedures." The total rate of operation of the shunt pathway thus calculated accounted for 4–8% of the uptake of mevalonate.

In livers perfused with [3-14C]mevalonate, the production of 14CO2 was lower than in livers perfused with [5-14C]- or [4,5-14C]mevalonate. This was expected since [3-14C]mevalonate yields [3-14C]acetocacetate which is partly converted by the liver to fatty acids, sterols, and CO2 (22).

In livers perfused with [2-14C]mevalonate, the measured rate of 14CO2 production (11.4–15.8%) of mevalonate uptake; not shown in Table I) corresponds to the sum of 14CO2 generated mostly in the sterol pathway (demethylation of lanosterol) and to a small extent via the shunt pathway (following oxidation of [2-14C]acetocacetate). The amount of 14CO2 generated in the sterol pathway can be calculated from the rate of [2-14C]mevalonate incorporation into squalene + sterols (see "Experimental Procedures"). By difference, one calculates the rate of 14CO2 production via the shunt pathway (reported in Table I). This rate, which is equivalent to 7–8% of the total rate of 14CO2 production, represents a small difference between two large numbers and is therefore inherently imprecise.

In the saponifiable extracts of livers from rats injected with large doses of [2-14C]mevalonate, Edmond and Popjak (2) had identified a fraction corresponding to [14C]farnesoate. This farnesoate (26) is generated from the oxidation of the corresponding prenol, farnesoic acid, which in turn derives from the hydrolysis of farnesyl pyrophosphate (27). We did not find any detectable [14C]farnesoate in the fatty acids extracted from livers perfused with physiological concentrations of [14C]mevalonate. When the methylated fatty acids were separated by gas-liquid chromatography, no peak was detected, and no label was collected at the retention time of methyl farnesoate. This was ascertained using reference standards of unlabeled and labeled methyl farnesoate synthesized as described by Cornforth and Popjak (21).

In addition, we attempted to identify labeled prenols by chromatographing the nonsaponifiable fraction on a column of aluminum oxide (21) followed by digitonin treatment of the squalene + sterol fraction. Less than 1.5% of the radioactivity of the nonsaponifiable fraction was found in the supernatant of the digitonides. It is likely that this small amount of radioactivity corresponds to sterols that are not precipitated by digitonin (28). We, therefore, conclude that under physiological concentrations of mevalonate, there is no significant production of long chain prenols and prenolates by the liver. These compounds are presumably generated when the sterol pathway is overloaded by large doses of mevalonate.

Incorporation of [5-14C]-, [4,5-14C]-, [3-14C]-, or [2-14C]mevalonate into the sterol pathway accounted for 70–98% of the uptake of mevalonate. Comparison between the rates of squalene + sterol synthesis from (i) exogenous [14C]mevalonate and (ii) 3H2O (expressed in mevalonate equivalents) reveals that the contribution of physiological levels of exogenous mevalonate to total mevalonate metabolism in the liver is very low (0.04–0.13%).

In order to test whether HMG-CoA (and thus acetyl-CoA) generated by the shunt pathway is mitochondrial or cytosolic, livers were perfused with either [4,5-14C]mevalonate or 100 mCi of 3H2O in the presence or absence of 2 mM {(-) hydroxycitrate. This inhibitor of ATP-citrate lyase (29) blocks the transfer via citrate of acetyl groups from the mitochondria to the cytosol, site of fatty acid synthesis. As we had shown previously (20), {(-) hydroxycitrate inhibited total fatty acid and squalene + sterol synthesis (measured by 3H incorporation) by 60%. It also inhibited the incorporation of [4,5-14C]mevalonate into n-fatty acids and squalene + sterols by 43% (from 16.4 ± 2.2 (n = 5) to 8.9 ± 1.3 (n = 5) pmol/g, dry weight × h; p < 0.01) and 24% (Table I: p = 0.06), respectively. On the other hand, {(-) hydroxycitrate did not affect the uptake of [4,5-14C]mevalonate, its conversion to 14CO2, and the total rate of mevalonate shunting.

One group of experiments was conducted to test the involvement of alcohol dehydrogenase, dimethylallyl alcohol, and dimethylallylactate in the mevalonate shunt pathway. Livers from male rats were perfused with 400 nm of [5-14C]mevalonate and either 20 mM ethanol, 0.05 mM 4-methylpyrazole, 5 mM dimethylallyl alcohol or 5 mM dimethylallylactate. The rates of mevalonate uptake and of squalene + sterol synthesis from exogenous mevalonate were not affected by any of the tested compounds, but the rate of [5-14C]mevalonate incorporation into 14CO2 was significantly decreased.

All the experiments described above were conducted in livers from fed rats perfused with a sterol-free medium containing 1.5 mM glucose to stimulate mevalonate synthesis. In order to test the influence of a decreased supply of mevalonate on the distribution of the substrate between the shunt and the sterol pathways, one group of livers was perfused with [5-14C]mevalonate and 75 μM Mevinolin, a potent inhibitor of HMG-CoA reductase (30). As expected, livers perfused with Mevinolin did not release mevalonate into the perfusate. Contrary to expectations, addition of Mevinolin led to a 39% decrease in the rate of mevalonate uptake by the liver. This is in contrast to what was observed in the perfused kidney (12) where Mevinolin increased the uptake of mevalonate by 30%. The rate of operation of the sterol pathway, measured by the incorporation of 3H2O, was 2730 and 61 nmol of mevalonate equivalent/g, dry weight × h in control and Mevinolin-treated livers, respectively. The corresponding rates of exogenous mevalonate incorporation were 3.5 and 1.2 nmol/
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Livers from fed rats were perfused with medium containing 15 mM glucose. Labeled mevalonate (MVA) was added as a bolus at 30 min. Metabolism of mevalonate via the sterol pathway represents incorporation into squalene + sterols. Metabolism of mevalonate via the shunt pathway is calculated from the production of $^{14}$CO$_2$ (see under "Experimental Procedures" and Footnote a). Rates are expressed either as picomoles of exogenous mevalonate/g, dry weight $\times$ h (mean $\pm$ S.E.), or as a percentage of the uptake of exogenous mevalonate.

**DISCUSSION**

In perfusions with [5-$^{14}$C]- or [4,5-$^{14}$C]mevalonate, the rate of $^{14}$CO$_2$ production amounted to the same fraction (1%) of the rate of mevalonate uptake by the liver. Since [5-$^{14}$C]- and [4,5-$^{14}$C]mevalonate yield $^{14}$CO$_2$ via [1-$^{14}$C]- and [1,2-$^{14}$C]acetyl-CoA, respectively, the yield of label from C-1 and C-2 acetyl-CoA must be practically the same. In these livers from fed rats perfused with 15 mM glucose, the tricarboxylic acid cycle does not therefore operate as a synthetic pathway (25). In other words, there is no significant influx into the cycle of unlabeled carbon which leaves the cycle carrying a fraction of the label of acetyl-CoA. 

(-)-Hydroxycitrate inhibits about equally the incorporation into $n$-fatty acids of [4,5-$^{14}$C]mevalonate and of labeled leucine (31, 32). This strongly suggests that acetyl-CoA derived from mevalonate is mitochondrial. Acetyl-CoA is transferred to the cytosol mostly (75%) via citrate and ATP-citrate lyase (29) and, to a minor extent (15%), via acetoacetate and cytosolic acetoacetyl-CoA synthetase (22). In the presence of (-)-hydroxycitrate, the inhibition of the citrate cleavage pathway is in part compensated by an activation of the transfer of acetyl groups via acetoacetate (22). In the presence of (-)-hydroxycitrate, the inhibition of the citrate cleavage pathway would have decreased the dilution of the specific activity of cytosolic acetyl-CoA by unlabeled acetyl groups transferred from the mitochondria. It appears that the shunt pathway of mevalonate metabo-

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**Table I**

Metabolism of mevalonate in the isolated perfused rat liver

| Labeled MVA (nm) | MVA (nm) | Sex | Addition to Perfusate | $^{14}$C $\text{MVA}$ Uptake | $^{14}$C $\text{Sterol Synthesis}$ | $^{14}$C $\text{CO}_2$ Production $\%$ | $^{14}$CO$_2$ in the Shunt $\%$ | Total Shunt $\%$ |
|-----------------|---------|-----|----------------------|-----------------------------|--------------------------------|-----------------|-----------------|-----------------|
| [4,5-$^{14}$C]  | 250     | M(5)|                      | 2486$\pm$8.4                | 50.2$\pm$3.1                 | 1.1$\pm$0.1    | 5.16$\pm$2.1   | 154$\pm$34      | 5.2             |
| [4,5-$^{14}$C]  | 250     | F(5)|                      | 2407$\pm$9.9                | 83.4$\pm$4.5                 | 1.2$\pm$0.4    | 128$\pm$24     | 5.3             |
| [4,5-$^{14}$C]  | 250     | M(5)| 2 mM (-)-Hydroxycitrate| 2836$\pm$24.0               | 70.5$\pm$7.8                 | 26.4$\pm$2.8   | 113$\pm$0.9    | 166$\pm$18      | 5.9             |
| [5-$^{14}$C]    | 400     | M(5)|                      | 6257$\pm$0.2                | 77.6$\pm$4.2                 | 59.0$\pm$3.3   | 1.05$\pm$0.7   | 372$\pm$33      | 5.9             |
| [5-$^{14}$C]    | 400     | F(5)|                      | 5691$\pm$9.7                | 82.5$\pm$3.1                 | 44.5$\pm$5.5   | 0.78$\pm$0.5   | 240$\pm$30      | 4.2             |
| [5-$^{14}$C]    | 400     | M(5)| 20 mM Ethanol         | 4424$\pm$400                | 60.5$\pm$5.7                 | 26.3$\pm$5.3   | 0.64$\pm$0.7   | 20.6$\pm$30     | 4.2             |
| [5-$^{14}$C]    | 400     | M(4)| 50 mM 4-Methylpyrazole| 4984$\pm$624                | 50.3$\pm$7.0                 | 28.9$\pm$4.0   | 0.60$\pm$1.0   | 15.4$\pm$36     | 4.2             |
| [5-$^{14}$C]    | 400     | M(5)| 5 mM Dimethylallyl Alcohol| 4921$\pm$608                | 50.3$\pm$7.0                 | 23.0$\pm$4.1   | 0.64$\pm$0.5   | 20.6$\pm$30     | 4.2             |
| [5-$^{14}$C]    | 400     | M(5)| 5 mM Dimethylacrylate | 4645$\pm$604                | 57.5$\pm$7.5                 | 25.4$\pm$2.5   | 0.57$\pm$0.6   | 20.6$\pm$30     | 4.2             |
| [5-$^{14}$C]    | 400     | M(5)| 75 mM Mevinolin       | 3425$\pm$122                | 49.0$\pm$8.9                 | 127$\pm$1.2    | 3.72$\pm$1.9   | 127$\pm$1.2     | 4.2             |
| [2-$^{14}$C]    | 250     | M(5)|                      | 2705$\pm$494                | 80.3$\pm$8.8                 | 116$\pm$21    | 10.5$\pm$1.3   | 110$\pm$11      | 3.8$\pm$1       |
| [2-$^{14}$C]    | 250     | F(5)|                      | 2431$\pm$467                | 73.8$\pm$6.3                 | 119$\pm$21    | 10.7$\pm$1.3   | 110$\pm$11      | 4.3$\pm$1       |
| [2-$^{14}$C]    | 300     | M(6)|                      | 4755$\pm$491                | 70.5$\pm$6.1                 | 30.9$\pm$4.0   | 0.71$\pm$0.15  | 195$\pm$29      | 4.1             |
| [2-$^{14}$C]    | 400     | F(6)|                      | 5646$\pm$696                | 78.7$\pm$9.5                 | 23.9$\pm$3.9   | 0.44$\pm$0.4   | 125$\pm$21      | 2.3             |

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The total rate of shunting, defined as the production of acetyl-CoA from exogenous mevalonate, is calculated by correcting the measured production of $^{14}$CO$_2$ for (i) the recovery of a tracer of NaH$^{14}$CO$_3$ (63 and 73% in livers from males and females, respectively), and (ii) the yield of label from mitochondrial acetyl-CoA to $^{14}$CO$_2$ in liver, determined in separate experiments from the differential yield in $^{14}$CO$_2$ from tracer of [l-$^{14}$C]- and [2-$^{14}$C]acetoacetyl-CoA (55% for both males and females (19)). These factors, which are not affected by (-)-hydroxycitrate, were not determined for perfusions with Mevinolin, ethanol, 4-methylpyrazole, dimethylallyl alcohol, and dimethylacrylate.

Significantly different from the corresponding control ($p \leq 0.05$ using two-sided t-test).

In experiments with [2-$^{14}$C]mevalonate, a large amount of $^{14}$CO$_2$ is generated in the sterol pathway. Production of $^{14}$CO$_2$ in the shunt, which accounts for a small fraction of the total production, is inherently imprecise. This is indicated by the presence of brackets around the rates of $^{14}$CO$_2$ production and the derived rates.

g, dry weight $\times$ h. Therefore, the fractional contribution of exogenous mevalonate to total squalene + sterols, and presumably to total mevalonate metabolism, was 0.13 and 2% in control and Mevinolin-treated livers, respectively.
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lism begins in the extramitochondrial space and ends in the mitochondria. Alcohol dehydrogenase which catalyzes the oxidation of dimethylallyl alcohol to \( \beta \)-methylcrotonaldehyde is a cytosolic enzyme. On the other hand, the steps >-methylcrotonyl-CoA to HMG-CoA, common with the leucine catabolism pathway, are mitochondrial. Therefore, the shunt pathway crosses the mitochondrial membrane at the level of either \( \beta \)-methylcrotonaldehyde or \( \beta \)-methylcrotonate. It has been shown (33) that ethanol is converted to acetaldehyde in liver cytosol, while acetaldehyde is oxidized to acetate mostly in the mitochondria. It is, therefore, likely that \( \beta \)-methylcrotonaldehyde is the intermediate of the shunt pathway that crosses the mitochondrial membrane.

The production of \( ^1{C}_4\text{CO}_2 \) from \([5-^{14}\text{C}]\text{mevalonate}\) was significantly decreased by compounds that (i) inhibit alcohol dehydrogenase (4-methylpyrazole), (ii) increase the cytosolic [NADH]/[NAD\(^+\)] ratio (ethanol and dimethylallyl alcohol), and (iii) dilute the specific activity of the shunt products (dimethylallyl alcohol and dimethylacrylate). Conversion factors needed to convert the production of \( ^{14}\text{CO}_2 \) to production of \([1-^{14}\text{C}]\text{acetyl-CoA}\) were not determined for perfusions in the presence of these substrates. Nevertheless, taken as a whole, the data of these experiments are compatible with the involvement of alcohol dehydrogenase, dimethylallyl alcohol, and dimethylacrylate in the shunt pathway.

The 68% inhibition by Mevinolin of the incorporation of \([5-^{14}\text{C}]\text{mevalonate}\) into sterols, also observed in the perfused kidney (12), stems probably from an inhibition of squalene synthesis by the fairly high concentration of inhibitor used (75 \( \mu \text{M} \)). This secondary site of action of Mevinolin is inferred by analogy with the effect of its analog Compactin (34). The actual inhibition of the incorporation of mevalonate into squalene + sterols is actually much greater than 68% since Mevinolin, by inhibiting the production of endogenous mevalonate, increased the specific activity of intracellular mevalonate.

The fractional contribution of exogenous mevalonate to total mevalonate metabolism is 0.13 and 2.0% in control and Mevinolin-treated livers. Therefore, the ratio of these percentages, i.e. 2.0 + 0.13 = 15, represents the increase in the specific activity of intracellular mevalonate induced by Mevinolin. This increase in specific activity probably accounts for the 2.3-fold increase in \( ^{14}\text{CO}_2 \) production induced by Mevinolin.

When a liver is perfused with physiological concentrations of labeled mevalonate, the contribution of the latter to total hepatic metabolism of the substrate is very low (less than 0.13%). Even when the rate of operation of the sterol pathway is 98% inhibited by Mevinolin, exogenous mevalonate contributes only 2% to the residual 2% of sterol synthesis. From the total rate of shunting of exogenous [4,5-\(^{14}\text{C}]\text{mevalonate}\) (154 pmol/g, dry weight \( \times \) h) and the fractional contribution of exogenous mevalonate to total mevalonate metabolism in the liver (0.13%), one calculates that the total rate of shunting of endogenous mevalonate is 118 nmol/g, dry weight \( \times \) h. In kidney (12), the corresponding rate is 40 times lower (2.89 nmol/g, dry weight \( \times \) h). Since the weight of the liver is about five times that of two kidneys, the amount of mevalonate shunted in the liver is about 200 times greater than in the kidneys. Since the liver accounts for at least half of the total production of mevalonate in the rat, one concludes that the liver is the main site of the shunt pathway of mevalonate metabolism.

In order to derive meaningful rates of mevalonate metabolism in the liver from the use of labeled mevalonate, one must determine the fractional contribution of the exogenous substrate to total mevalonate metabolism in the liver. This contribution can be assessed from the incorporation into sterols from \( ^{14}\text{O}_2 \) added to the perfusate. One should recognize that any error in the determination of this very small contribution can lead to a large error in the estimate of the absolute rates of mevalonate metabolism through pathways other than the sterol pathway.

The very small fractional contribution of perfusate \([^{14}\text{C}]\text{mevalonate}\) to total mevalonate metabolism in the liver implies that, inside the liver cell, the specific activities of mevalonate are three orders of magnitude lower than outside the cell. It follows that one should not be able to derive the turnover rate of whole body mevalonate from the turnover rate of a tracer of labeled mevalonate injected into the plasma. In 250-g rats, the whole body rate of squalene + sterol synthesis (measured by \( ^{14}\text{O}_2 \) incorporation) is equivalent to about 2400 nmol of mevalonate equivalent/kg \( \times \) min. The pool of extracellular mevalonate (7) is 0.15 nmol/ml \( \times \) 200 ml/kg = 30 nmol/kg. Since the half-life of plasma mevalonate in the rat is about 9 min (7), the turnover rate of extracellular mevalonate is \( (2.31 + 2400) \times 100 = 0.1\% \) of the whole body mevalonate turnover. Parker et al. (35) have recently shown that in humans given a constant infusion of \( [5-^{3}\text{H}]\text{mevalonate}\), the turnover of plasma mevalonate also accounts for about 0.1% of the turnover of whole body mevalonate measured by sterol balance.

Lakshmanan and Veech (36) have attempted to shut off the endogenous rate of mevalonate production by injecting large amounts of \([^{14}\text{C}]\text{mevalonate} + ^{1}\text{H}_2\text{O}\) intravenously into rats. From the evolution of the \(^{2}\text{H}/^{14}\text{C}\) ratio in sterol livers, as a function of the dose of mevalonate injected, they concluded that one has to inject 1.0–5 mmol of \( R-[^{3}\text{H}]\text{mevalonate} \) of rat to block endogenous mevalonate production. In other words, the pool of extracellular mevalonate had to be increased more than 100-fold in order to equilibrate the specific activities of intra- and extracellular mevalonate in the liver.

From the above considerations, one can conclude that it is impossible to quantitate under physiological in vivo conditions the rate of operation of the shunt or of the sterol pathway using tracers of radioactive mevalonate. When such tracers are injected in vivo, they essentially bypass the liver which is the main site of operation of both the shunt and the sterol pathways. These in vivo protocols amount to little more than an intrarenal infusion of the tracer. Since the kidney accounts for less than 0.1% of the metabolism of mevalonate in the body, these experiments cannot yield any valid quantitative data on the metabolism of mevalonate in vivo.

In isolated cell or organ preparations, tracers of \([^{14}\text{C}]\text{mevalonate}\) can be used in conjunction with \( ^{1}\text{H}_2\text{O} \) to measure the rate of operation of the shunt pathway, since the ratio of incorporation of \(^{2}\text{H}\) and \(^{14}\text{C}\) into sterols yields the fractional contribution of extracellular mevalonate to total mevalonate metabolism in this organ. This approach cannot be used in vivo since the production of \(^{14}\text{CO}_2 \) reflects mostly the metabolism of plasma mevalonate in the kidney.

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