Evidence for an underestimation of the shunt pathway of mevalonate metabolism in slices of livers and kidneys from fasted rats and rats in diabetic ketosis

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Abstract Yields of $^{14}CO_2$ from [2-^{14}C]mevalonate and [5-^{14}C]mevalonate have been used by others to estimate the activity of the non-sterol-forming pathway, also called the mevalonate shunt pathway, and yields of ^{14}C in sterols have been used to estimate the activity of the sterol-forming pathway. Both these pathways operate following the conversion of carbon 1 of mevalonate to CO_2. The estimations of the shunt pathway contribution are dependent upon the fractions of carbons 2 and 5 of mevalonate that are oxidized to CO_2 in the Krebs cycle after leaving the pathway. Unless all of carbons 2 and 5 are oxidized to CO_2, the estimates are minimal. The metabolism of mevalonate has now been examined in slices of livers and kidneys from fasted rats and rats in diabetic ketosis. Yields of $^{14}CO_2$ from [1-^{14}C]mevalonate are used as the measure of the contributions of all the pathways by which carbon 1 of mevalonate is converted to CO_2. Yields of ^{3}H-labeled nonsaponifiable lipids from [5-^{3}H]mevalonate are used as the measure of the sterol-forming pathway. The differences in these yields are then taken as the measure of the non-sterol-forming pathway or pathways. Yields of $^{14}CO_2$ from [1-^{14}C]mevalonate markedly exceeded the sum of the yields of ^{14}C in CO_2 and nonsaponifiable lipids from either [2-^{14}C]mevalonate or [5-^{14}C]mevalonate. Therefore, in liver and kidney, under the conditions of this study, either one or more pathways other than the shunt pathway, by which mevalonate can be metabolized to other than sterols, is operative to a marked degree, or estimates of the shunt pathway's contributions as judged by yields of $^{14}CO_2$ from [2-^{14}C]mevalonate and [5-^{14}C]mevalonate are significantly underestimated.—**Brady, P. S., W. C. Schumann, S. Ohgaku, R. F. Scofield, and B. R. Landau.** Evidence for an underestimation of the shunt pathway of mevalonate metabolism in slices of livers and kidneys from fasted rats and rats in diabetic ketosis. *J. Lipid Res.* 1982. **23:** 1317–1320

Supplementary key words mevalonate shunt pathway • sterols • kidney • liver

Mevalonate is metabolized by a sterol and a non-sterol-forming pathway, the latter also called the mevalonate shunt pathway (1). The branch point for these pathways is formed following the conversion of carbon 1 of mevalonate to CO_2. The remaining five carbons of mevalonate are incorporated into sterols in the sterol-forming pathway. The carbons of mevalonate are converted to HMG CoA via the shunt pathway, which is then cleaved to acetoacetic acid and acetyl CoA. These can be oxidized to CO_2 in the Krebs cycle.

Quantitation of mevalonate metabolism by these pathways has been made from yields of $^{14}CO_2$ and ^{14}C-labeled sterols from [2-^{14}C]mevalonate and [5-^{14}C]mevalonate (2). Of the mevalonate carbons converted to squalene, one-sixth of those that were in position 2 of mevalonate are oxidized to CO_2 in the conversion of squalene to cholesterol. Carbon 5 from mevalonate is completely retained in sterol formation. Since $^{14}CO_2$ formed from [5-^{14}C]mevalonate and not [2-^{14}C]mevalonate is then the direct measure of the shunt's activity, the former has been used for most estimations (2–7). When [2-^{14}C]mevalonate has been used, the yield of $^{14}CO_2$ calculated to have been formed in the sterol-forming pathway has been subtracted from the total amount of $^{14}CO_2$ formed to give a measure of the shunt pathway. However, as noted by Righetti et al. (2), estimates of shunt activity based on these $^{14}CO_2$ yields still represent only minimum values. This is so unless all the carbon 2 of mevalonate incorporated into acetoacetic acid and all of carbon 5 incorporated into acetyl CoA via the shunt pathway are oxidized to CO_2.

On the basis of studies using [2-^{14}C]mevalonate and [5-^{14}C]mevalonate it has been concluded that the kidney metabolizes mevalonate by the shunt pathway to a much

Abbreviations: HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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greater extent than other tissues (2). The pathway appears, from these estimates, to be more active in female than in male rats (3) and humans (4), to be influenced by pregnancy (5) and perhaps thyroid state (6), and to be depressed in streptozotocin-induced diabetic rats in ketosis (7).

The purpose of the present study was to estimate the contributions of the non-sterol-forming pathway(s) to mevalonate metabolism by a method independent of the fraction of acetyl CoA metabolized in the Krebs cycle, and then to compare these estimates with those made from yields of ^{14}C from [2-^{14}C]mevalonate and [5-^{14}C]mevalonate. This has been done in liver and kidney slices from fasted rats and rats in diabetic ketosis. We have used the yield of $^{14}CO_2$ from [1-^{14}C]mevalonate as the measure of mevalonate metabolism by all pathways and the incorporation of label from carbon 5 of mevalonate into nonsaponifiable lipids as the measure of the sterol-forming pathway. The difference in these yields is then the measure of the non-sterol-forming pathway(s).

EXPERIMENTAL PROCEDURES

Materials

Female rats of the Sprague-Dawley strain, weighing 200–300 g, were used; they were fed ad libitum. Diabetes was induced in the rats by the intravenous injection of streptozotocin after the rats were fasted for 24 hr. The diabetes was then controlled with insulin for 9 to 11 days (8). A rat was used only if it developed marked ketosis along with glucosuria after insulin was withdrawn. At killing, the concentration of glucose in blood from the rats averaged 27.9 mM (9) and the concentration of hydroxybutyrate averaged 5.6 mM (10).

R,S-[1-^{14}C]Mevalonolactone (6 μCi/μmol) was purchased from Amersham Corp., Arlington Hts., IL, sodium R,S-[5-^{14}C]mevalonate (15 μCi/μmol) was from Research Products International Corp., Mount Prospect, IL, and R,S-[2-^{14}C]mevalonolactone (17 μCi/μmol) and R,S-[5-^{3}H]mevalonolactone (7.8 μCi/μmol) were from New England Nuclear Corp., Boston, MA. In addition to the evidence for purity provided by their manufacturers, the lactones were subjected to thin-layer chromatography on silica gel plates developed in absolute ethanol–toluene 1:4 (by volume). Each gave a single spot containing more than 99% of the ^{14}C applied. Each was hydrolyzed with NaOH to its sodium salt (11) just before use. There is good evidence that in the rat the R-isomer of mevalonate, but not the S-isomer, is metabolized (12).

Methods

Incubations were essentially as described by Righetti et al. (2). Livers and cortex of kidneys from the rats, fasted for 24 hr or in diabetic ketosis, were sliced using a Stadie-Riggs slicer. About 1 g of slices in 10 ml of Krebs-bicarbonate buffer containing 0.1 mM mevalonate in a 125-ml flask were incubated for 90 min at 37°C with shaking. The contents of the flask were gassed for the first 10 min of the incubation period with 95% O_2–5% CO_2 saturated with water at 37°C. The flask was then sealed and the incubation was continued for the additional 80 min. In each experiment, there were four or six such flasks. Slices from the kidneys of two or three rats were sequentially distributed to half these flasks and slices from the livers of the rats were distributed sequentially to the remaining flasks. One flask with slices of kidneys and one with slices of livers contained [1-^{14}C]mevalonate (2.2 to 5.6 μCi per flask) and [5-^{3}H]mevalonate (4.0 to 19.9 μCi per flask; in a second pair of flasks, [2-^{14}C]mevalonate (4.0 to 7.5 μCi per flask) was present. When there was a third pair of flasks they contained [5-^{14}C]mevalonate (1.7 to 14.7 μCi per flask) with [5-^{3}H]mevalonate (5.4 to 24.4 μCi per flask). Incubation was terminated by the addition of sulfuric acid. The CO_2 that evolved in each flask was assayed for ^{14}C following its absorption into Beckman Bio-Solv III counting fluid contained in a scintillation vial suspended within the flask. Identical incubations, except without tissue, were also conducted and negligible $^{14}CO_2$ was evolved.

The slices were digested in alcoholic KOH and the digest was extracted three times with petroleum ether. The petroleum ether extract, i.e., the nonsaponifiable lipid fraction, was washed with water until the washings had negligible counts. An aliquot of the extract was evaporated and the resulting residue was combusted to CO_2 and water (using a Model 306 Oxidizer, Packard Instrument Co., Inc., Downers Grove, IL). These were assayed for ^{14}C and ^{3}H. There was negligible ^{14}C from [1-^{14}C]mevalonate in the petroleum ether extract.

RESULTS AND DISCUSSION

Table 1 records the incorporations of isotope into CO_2 and nonsaponifiable lipids from the specifically labeled mevalonates incubated with the slices of kidney and liver. There is no apparent difference between the results obtained with slices from rats fasted 24 hr and those obtained with slices from rats in diabetic ketosis. However, there is considerable variation from experiment to experiment. This also characterizes the exper-

TABLE 1. Incorporation from specifically labeled mevalonates into CO_2 and nonsaponifiable lipids by slices of kidney and livers from normal rats fasted 24 hr and rats in diabetic ketosis[a]

Tissue–Condition	Expt. No.	$^{14}CO_2$ from Mevalonate			Incorporation of Nonsaponifiable Lipids from Mevalonate				Difference in Yields from 1-^{14}C and		Non-Sterol Pathway(s)
		1-^{14}C	2-^{14}C	5-^{14}C	2-^{14}C	5-^{14}C	5-^{3}H[b]	5-^{3}H[c]	2-^{14}C[d]	5-^{14}C[e]	Estimate[f]
Kidney–Fasted	1	190.2	21.3	26.6	69.5	49.6	43.3	58.0	99.4	114.0	132.2
	2	122.0	14.8		34.9			45.2	72.3		76.8
	3	99.7	16.5	15.3	32.7	26.2	24.3	26.9	50.5	58.2	72.8
	4	152.0	21.3	36.4	46.3	46.6	52.2	61.2	84.4	69.0	90.8
Kidney–Ketotic	1	161.6	20.3		75.4			56.9	65.9		104.7
	2	102.6	5.4		35.4			38.5	61.8		64.1
	3	172.5	13.9	18.1	83.9	102.0	69.7	47.9	74.7	52.4	124.6
Liver–Fasted	1	106.1	11.7	2.1	63.1	36.6	39.7	55.4	31.3	67.4	50.7
	2	71.2	5.5		22.8			38.3	42.9		32.9
	3	45.3	7.0	1.3	18.7	20.1	18.4	16.1	19.6	23.9	29.2
	4	87.3	12.9	3.3	38.2	55.6	49.1	55.1	36.2	28.4	32.2
Liver–Ketotic	1	72.7	11.3		52.4			43.7	9.0		29.0
	2	90.6	2.4		36.4			33.3	51.8		57.3
	3	89.9	14.6	3.7	60.0	58.3	41.2	55.1	15.3	27.9	24.8

[a] Incorporation is expressed as nmol/g of slices per hr. Calculations have been made from the yields of $^{14}CO_2$ during 80 min and the incorporation into lipids during 90 min of incubation (see Methods).
[b] Incubated with [5-^{14}C]mevalonate.
[c] Incubated with [1-^{14}C]mevalonate.
[d] Yields of ^{14}C in CO_2 and sterols from [2-^{14}C]mevalonate subtracted from the $^{14}CO_2$ yield from [1-^{14}C]mevalonate.
[e] Yields of ^{14}C in CO_2 and sterols from [5-^{14}C]mevalonate substracted from the $^{14}CO_2$ yields from [1-^{14}C]mevalonate.
[f] Yields of ^{3}H in sterol from [5-^{3}H]mevalonate incubated with [1-^{14}C]mevalonate substracted from the $^{14}CO_2$ yields from the [1-^{14}C]mevalonate.

iments of Righetti et al. (2). Yields of $^{14}CO_2$ from [1-^{14}C]mevalonate exceeded those from [2-^{14}C]mevalonate and [5-^{14}C]mevalonate with the lowest amounts of $^{14}CO_2$ being formed from [5-^{14}C]mevalonate by liver. $^{14}CO_2$ yields are net yields, the difference between the quantities of $^{14}CO_2$ formed from the ^{14}C-labeled mevalonate and the quantities reincorporated through $^{14}CO_2$ fixation.

CO_2 is formed from carbon 1 of every molecule of mevalonate metabolized to isopentenyl pyrophosphate, the branch point for the shunt and sterol-forming pathways. The yield of $^{14}CO_2$ from [1-^{14}C]mevalonate is then the measure of the sum of the non-sterol- and sterol-forming pathways, as well as any other non-sterol-forming pathway that may exist in which CO_2 is formed from carbon 1. Incorporation of ^{14}C from [5-^{14}C]mevalonate is a reliable measure of the sterol-forming pathway, since carbon 5 is retained in the formation of sterols (2, 12). The hydrogens bound to carbon 5 are also retained except for one hydrogen removed in the cyclization of squalene and one in the formation of the double bond in Ring B of cholesterol (13). Thus, incorporation of ^{3}H from [5-^{3}H]mevalonate into sterols should be about the same as incorporation of ^{14}C from [5-^{14}C]mevalonate. When [5-^{14}C, 5-^{3}H]mevalonate was incubated this proved to be the case (Table 1). Incubation of [1-^{14}C]mevalonate and [5-^{3}H]mevalonate together then permits an estimation of the total metabolism of mevalonate to isopentenyl pyrophosphate and its metabolism to sterols.

If the shunt were the only pathway, other than the sterol pathway, by which mevalonate is metabolized, the yield of $^{14}CO_2$ from [1-^{14}C]mevalonate should equal the sum of the yields of $^{14}CO_2$ from [5-^{14}C]mevalonate and of ^{14}C into nonsaponifiable lipids from [5-^{14}C]mevalonate, or the equivalent, ^{3}H from [5-^{3}H]mevalonate. This assumes the yields of $^{14}CO_2$ and ^{14}C-labeled nonsaponifiable lipids from [5-^{14}C]mevalonate are good measures of the pathways (2). As can be seen from the next to last column of Table 1, the yields from [1-^{14}C]mevalonate exceed the sum of the yields from [5-^{14}C]mevalonate. As shown in the prior column, the sums of the incorporations of ^{14}C from [2-^{14}C]mevalonate into CO_2 and nonsaponifiable lipids, also used as measures of the pathways (2), are also much less than the yields of $^{14}CO_2$ from [1-^{14}C]mevalonate. The last column shows the differences between the yields of $^{14}CO_2$ from [1-^{14}C]mevalonate and the yields of nonsaponifiable lipids from [5-^{3}H]mevalonate. These differences are then a measure of the non-sterol-forming pathway(s). They are for kidney 3 to 7 times greater than the yield of ^{14}C from [5-^{14}C]mevalonate and for liver 7 to 24 times greater (last column compared to the fifth column of Table 1).

The yields from [1-^{14}C]mevalonate thus indicate larger contributions by the shunt pathway than calculated by the method of Righetti et al. (2) or contributions by another non-sterol-forming pathway or pathways by which $^{14}CO_2$ is formed from [1-^{14}C]mevalonate. In fasting and diabetic ketosis sterol formation is small, and in mammalian tissues isopentenyl pyrophosphate is the precursor of several substances besides sterols (14). Their formation could account for a significant portion of the $^{14}CO_2$ yield from [1-^{14}C]mevalonate. There remains the considerable evidence, based in part on studies of nephrectomized rats (3, 15), that the kidney and not the liver is the major site of shunt activity. Non-sterol-forming pathway(s) other than the shunt might then be more evident in liver than kidney.

It is not surprising that contributions estimated using [1-^{14}C]mevalonate exceed those obtained using [2-^{14}C]mevalonate or [5-^{14}C]mevalonate, since only a fraction of ^{14}C from the latter when incorporated into HMG CoA, the intermediate in the shunt, would be expected to be oxidized to $^{14}CO_2$. This would especially be the case for liver from an animal in a ketotic state, since much of the carbon of the HMG CoA would then be expected to be converted to ketone bodies and not CO_2. This could be the explanation for the lower yield of $^{14}CO_2$ from [5-^{14}C]mevalonate administered to rats in diabetic ketosis when compared to the normal rat, rather than a depression from normal in the shunt in diabetic ketosis (7). Thus, using the yields of $^{14}CO_2$ from [5-^{14}C]mevalonate as the measure of the pathway in one condition compared to another may be misleading, since, for the comparison to be valid, the same fraction of ^{14}C from ^{14}C-acetyl CoA formed via the pathway must be converted to $^{14}CO_2$ in both conditions.

This research was supported by Grant AM-14507 from the National Institutes of Health and a grant from the Diabetes Association of Greater Cleveland. Dr. Paul S. Brady is a postdoctoral fellow supported by Training Grant AM-07319 from the National Institutes of Health.

Manuscript received 23 February 1982 and in revised form 21 July 1982.

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