Role of the kidneys in the metabolism of plasma mevalonate. Studies in humans and in rhesus monkeys.

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Research Article

Studies were carried out in humans and in rhesus monkeys to determine the role of the kidneys in the metabolism of circulating mevalonic acid (MVA). Following intravenous infusion of [14C]MVA and [3H]cholesterol, there was a rapid appearance of [14C]squalene in the kidneys that exhibited a significantly longer half-life than plasma or hepatic squalene. In man and in rhesus monkeys there was a rapid equilibration between newly synthesized cholesterol from MVA and exogenously administered cholesterol in all tissues except the kidneys, where the specific activity ratio of newly synthesized to exogenous cholesterol was significantly higher. Estimates of the quantitative metabolism of intravenously infused radiolabeled MVA in the monkey demonstrated that 23% was excreted in the urine, 67% metabolized to cholesterol (58% in nonrenal tissues and 9% in the kidneys), and 10% catabolized to CO2 and nonsteroid products. Measurements of MVA metabolism in anephric and uninephric patients demonstrate that, in the absence of renal uptake of MVA, exogenous and newly synthesized cholesterol achieve almost instantaneous equilibrium in the plasma; whereas in control subjects with normal renal function, this equilibration required at least 21 d for the two cholesterol decay curves to become parallel. These results suggest that the kidneys are solely responsible for the observed disequilibrium between newly synthesized and exogenous cholesterol; we suggest that this was due to the delayed release […]

Role of the Kidneys in the Metabolism of Plasma Mevalonate

Studies in Humans and in Rhesus Monkeys

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Abstract

Studies were carried out in humans and in rhesus monkeys to determine the role of the kidneys in the metabolism of circulating mevalonic acid (MVA). Following intravenous infusion of [^{14}C]MVA and [^{3}H]cholesterol, there was a rapid appearance of [^{14}C]squalene in the kidneys that exhibited a significantly longer half-life than plasma or hepatic squalene. In man and in rhesus monkeys there was a rapid equilibration between newly synthesized cholesterol from MVA and exogenously administered cholesterol in all tissues except the kidneys, where the specific activity ratio of newly synthesized to exogenous cholesterol was significantly higher. Estimates of the quantitative metabolism of intravenously infused radiolabeled MVA in the monkey demonstrated that 23% was excreted in the urine, 67% metabolized to cholesterol (58% in nonrenal tissues and 9% in the kidneys), and 10% catabolized to CO_2 and nonsteroid products.

Measurements of MVA metabolism in anephric and uninephric patients demonstrate that, in the absence of renal uptake of MVA, exogenous and newly synthesized cholesterol achieve almost instantaneous equilibrium in the plasma; whereas in control subjects with normal renal function, this equilibration required at least 21 d for the two cholesterol decay curves to become parallel. These results suggest that the kidneys are solely responsible for the observed disequilibrium between newly synthesized and exogenous cholesterol; we suggest that this was due to the delayed release of newly synthesized cholesterol from the kidneys into the plasma compartment following intravenous infusion with radiolabeled MVA.

The data document the importance of the kidneys in the metabolism of circulating MVA. However, calculation of the quantative significance of this pathway in relation to whole body MVA metabolism indicates that renal metabolism of MVA accounts for ~0.1% of daily MVA turnover, and that alterations in this pathway due to any form of renal pathology would not result in significant changes in hepatic or whole body sterol synthesis rates. We urge caution in the use of radiolabeled MVA in long-term kinetic studies of sterol metabolism because our data show that the plasma compartment of MVA is not necessarily in isotopic equilibrium with tissue MVA.

Introduction

Mevalonic acid (MVA)[1] is an obligate precursor in the biosynthetic pathway of all polyisoprenoid compounds, including cholesterol, and is the product of the key regulatory, rate-limiting enzyme in sterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (1). Previous studies have demonstrated that MVA levels in plasma are regulated in vivo in animals (2) and in man (3), and radiolabeled MVA has been used extensively in kinetic studies of squalene (4), cholesterol (5), and bile acid (6) synthesis in man under a variety of clinical situations.

The use of radiolabeled MVA as a precursor for in vivo kinetic studies of sterol metabolism is complicated by the fact that intravenously administered MVA is utilized by a number of metabolic pathways: catabolism to CO_2 (7), cholesterol and bile acid synthesis (4, 6, 8), and urinary excretion (9, 10). In an attempt to correct for nonsteroid metabolism of intravenously administered [^{14}C]MVA, and thereby calculate the actual dose of radiolabeled MVA converted to cholesterol in squalene kinetic measurements, we coadministered [^{3}H]cholesterol and determined the fractional conversion of MVA to cholesterol using the plasma cholesterol isotope ratio (4, 8). Our studies indicated that the fractional conversion of MVA to cholesterol is variable but is directly related to the rate of whole body cholesterol synthesis in man (8). Previously, we had demonstrated that the decay kinetics of newly synthesized and intravenously administered cholesterol were in disequilibrium for the initial 21 d following intravenous administration, after which time the decay curves were parallel for as long as 1 yr (Fig. 2, reference 4). The forces contributing to this initial disequilibrium were not completely understood, but a number of animal studies suggested that renal metabolism of circulating MVA could be playing a role (11–14).

In the present study we have investigated the role of the kidneys in the metabolism of circulating MVA in humans and in rhesus monkeys. The data demonstrate that (*a*) plasma MVA is rapidly taken up by the kidneys and converted to squalene, (*b*) the radiolabeled squalene exhibits an extended residence time in the kidneys and is slowly converted to cholesterol, and (*c*) the renal cholesterol equilibrates with circulating plasma cholesterol; the overall process requires about 21 d. Calculations of plasma MVA concentration, turnover, and renal metabolism demonstrate that in the primate, as in the rodent, the kidneys are a significant catabolic site for

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1. *Abbreviations used in this paper:* HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; MVA, mevalonic acid.

circulating MVA but, since the concentration of plasma MVA and its turnover are quantitatively minor with respect to whole body MVA metabolism, this process appears to play an insignificant role in whole body MVA and cholesterol metabolism.

Methods

Materials. *R,S*-[5-^{14}C]MVA (12.2 mCi/mmol) was purchased from Schwarz/Mann, Orangeburg, NY, and [1,2-^{3}H]cholesterol (40 Ci/mmol) from New England Nuclear Corp., Boston, MA. [1,2-^{3}H]Cholesterol was purified by thin-layer chromatography on silica gel G developed in benzene:ethyl acetate, 4:1 (vol:vol), and the purified cholesterol eluted with absolute ethanol. Radioisotopic materials were prepared and infused as previously described (4, 8).

Patients (Table I). N.A. was a 59-yr-old caucasian female with type II hyperlipidemia who had previously undergone triple coronary bypass surgery. A history of angina and a family history of hypercholesterolemia and premature cardiovascular disease were consistent with a diagnosis of heterozygous familial hypercholesterolemia. 18 d post infusion of 250 μCi *R,S*-[5-^{14}C]MVA and 25 μCi [1,2-^{3}H]cholesterol for measurement of plasma squalene kinetics (4, 8), the patient died from a myocardial infarction. At autopsy she was found to have atherosclerosis of the aorta and coronary arteries, aortic stenosis, bilateral arcus and xanthomata of the Achilles tendon and extensor tendons of the hands. Tissue samples were obtained at autopsy for determination of squalene and cholesterol masses and specific activities. All analytical procedures were carried out within 1 mo of obtaining the biopsy samples.

Patients V.F. and T.J. (Table I) were surgically anephric and were maintained on dialysis thrice weekly. Immediately following dialysis each patient was intravenously infused with *R,S*-[5-^{14}C]MVA (~5 μCi) and [1,2-^{3}H]cholesterol (~5 μCi), and blood samples were drawn over the subsequent 21 d for measurement of cholesterol specific activities to determine the fractional conversion of MVA to cholesterol (8).

Other patients involved in this study (Table I) and the individual study protocols are described in Results. For comparative purposes we have included data in this report from 15 control subjects (i.e., normal renal function and routine measurements of MVA fractional conversions) previously described (4, 8). All patients gave informed consent to studies that had been reviewed and approved by the Rockefeller University Hospital Institutional Review Board.

Animals. Two adult female rhesus monkeys (*Macaca mulatta*) were fed Purina Primate Chow (Ralston Purina Co., St. Louis, MO) ad lib. In the first study monkey no. 1 was given *R,S*-[5-^{14}C]MVA (250 μCi) and [1,2-^{3}H]cholesterol (25 μCi) intravenously, and plasma and kidney samples were obtained after ketamine sedation on days 2, 4, and 8 post infusion. The animal was killed on day 15 for analysis of tissue squalene and cholesterol masses and specific activities.

In the second study monkey #2 was given a similar intravenous infusion of *R,S*-[5-^{14}C]MVA and [1,2-^{3}H]cholesterol and maintained under ketamine sedation to obtain serial biopsies of kidney, liver, muscle, and plasma at 2, 4, 6, and 8 h after infusion; urine and fecal samples were collected continuously over a 24-h period and the animal was killed 24 h post infusion. Tissues and carcass were assayed for squalene and cholesterol mass and radioactivity as described below.

Analytical procedures. Tissue and plasma squalene and cholesterol levels were measured in duplicate by the gas-liquid chromatographic method of Liu et al. (15). Squalene kinetics and MVA fractional conversion rates were quantitated as previously described (4, 8). Plasma MVA concentrations were assayed in protein-free ultrafiltrates prepared from freshly drawn heparinized blood by the enzymatic assay of Popjak et al. (2), as modified by Parker et al. (3). To facilitate comparisons of the various studies, specific activity data have been normalized as percent dose per gram cholesterol and percent dose per milligram squalene. Since only the *R*-form of MVA is converted to sterols, the dose-normalized specific activities of the ^{14}C-compounds were multiplied by two.

Results

Squalene and cholesterol synthesis from mevalonate in human tissues. During the course of studies on plasma squalene kinetics and MVA fractional conversion to cholesterol, patient N.A. died 18 d after intravenous infusion with *R,S*-[5-^{14}C]MVA and [1,2-^{3}H]cholesterol (patient no. 16 in reference 8). Tissue samples were obtained at autopsy, and squalene and cholesterol masses and specific activities measured. Tissue squalene and cholesterol concentrations (Table II) were similar to those previously reported by Liu et al. (15) in a randomly sampled population for all tissues, except that aortic plaque and tendons were significantly higher in cholesterol in this heterozygous familial hypercholesterolemic patient. Radiolabeled squalene was found predominantly in the kidneys; only trace amounts were found in the adipose, adrenal, and aortic wall tissues ($<4 \times 10^{-4}$ percent dose/mg) with no detectable levels seen in the other tissues tested. The presence of [^{14}C]squalene in the kidneys following intravenous administration of [^{14}C]MVA is consistent with data from studies in the rat where a significant portion of circulating MVA is taken up by and converted to squalene and cholesterol in this tissue (13, 14). The intravenously administered [^{3}H]cholesterol was evenly distributed

*Table I. Patient Characteristics**

Patients	Age	Sex	Weight	Plasma lipids‡		Comments
				Cholesterol	Triglyceride	
	yr		*kg*	*mg/dl*	*mg/dl*	
N.A.	59	F	46	301±36 (22)	193±53 (22)	HtFH
V.F.	52	F	49	164±9 (9)	131±26 (9)	Anephric
T.J.	30	M	50	147±6 (3)	189±21 (3)	Anephric
A.A.	53	M	83	384±43 (25)	1601±654 (25)	MHL, Uninephric
M.W.	30	M	81	161±28 (25)	108±26 (25)	CHD
B.K.	72	F	52	187±9 (25)	96±14 (25)	Normal

Abbreviations used in this table: HtFH, heterozygous familial hypercholesterolemic; MHL, mixed hyperlipidemic; CHD, coronary heart disease.
* In addition to the patients presented here, data from 15 previously reported patients with normal renal function (4, 8) have been used for comparative purposes. ‡ Data presented as mean±SD for (*n*) determinations.

throughout the body cholesterol pools as judged by the similarity in dose-normalized specific activity values found in the majority of tissues (0.74±0.03 percent dose/g cholesterol, $n = 21$); only aortic plaque and tendons (both Achilles and unaffected) had significantly lower [^{3}H]cholesterol specific activities (0.03–0.08 percent dose/g). Radiolabeled cholesterol arising from [^{14}C]MVA was also evenly distributed in most tissues and, in every tissue except kidney, had a pattern of distribution similar to that of the intravenously administered [^{3}H]cholesterol as judged by the $^{14}C/^3H$ isotope ratio of cholesterol specific activities (0.66±0.08 in 23 tissues, Table II). A totally different pattern was found in the kidneys where the [^{14}C]cholesterol specific activity was some 28-fold greater than that found in other tissues, and the isotope ratio of [^{14}C]cholesterol to [^{3}H]cholesterol also was significantly higher (20.69 vs. 0.66). The total radioactivity of [^{14}C]squalene plus [^{14}C]cholesterol in the kidneys 18 d after administration of [^{14}C]MVA accounted for 11.3% of the total dose of the *R*-form of the radiolabeled MVA.

The plasma cholesterol specific activity and isotope ratio changed significantly over the 18-d study period (Table III) with the relative proportion of ^{14}C-labeled cholesterol increasing steadily over the study period. Our previous studies demonstrated a similar pattern in all patients given [^{14}C]MVA and [^{3}H]cholesterol; a minimum of 21 d is required before the isotope ratio becomes constant and the two decay curves are parallel (4, 8). The autopsy data from patient N.A. would suggest that the progressive increase in the plasma [^{14}C]cholesterol to [^{3}H]cholesterol ratio and the delayed equilibration between the exogenous [^{3}H]cholesterol and the newly synthesized [^{14}C]cholesterol result from: (*a*) specific uptake of circulating MVA by the kidneys and a rapid conversion of MVA to squalene in renal tissues, (*b*) a delayed conversion in the kidneys of newly synthesized squalene to cholesterol, and/or (*c*) a delayed release of newly synthesized cholesterol from this tissue. To investigate these points further studies were carried out in rhesus monkeys.

Squalene and cholesterol synthesis from mevalonate in tissues of the rhesus monkey: 15-d study. To establish the time course of circulating MVA metabolism in body tissues, a female rhesus monkey was infused intravenously with *R,S*-[5-^{14}C]MVA and [1,2-^{3}H]cholesterol, and serial biopsies of kidney tissues were obtained on days 2, 4, and 8 after infusion. On day 15 the animal was killed and various tissues analyzed for squalene and cholesterol masses and specific activities.

The data presented in Table IV demonstrate the complete absence of plasma squalene radioactivity on day 2 and thereafter, which is consistent with its rapid turnover (4); however, significant amounts of radiolabeled squalene were found in the kidneys on day 2 and remain detectable up to day 15. These results are similar to those obtained from the autopsy of patient N.A. (Table II) and support the thesis that renal

Table II. Tissue Squalene and Cholesterol Mass and Specific Activity 18 d after Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol in Patient N.A.

Tissue	Squalene	Squalene	Cholesterol Mass	Cholesterol ^{3}H	Cholesterol ^{14}C	Ratio ($^{14}C/^3H$)
	*μg/g**	*% dose/mg‡*	*mg/g**	*% dose/g‡*	*% dose/g‡*	
Adipose	661.6	3.8×10^{-7}	1.2	0.37	0.20	0.54
Adrenal	17.6	4.1×10^{-4}	10.5	0.98	0.58	0.59
Aortic plaque	1.6	3.4×10^{-4}	27.6	0.07	0.05	0.71
Aortic wall	18.6	0	4.3	0.30	0.19	0.63
Bile	0.2	0	5.5	0.58	0.42	0.72
Colon	40.1	0	1.4	0.72	0.44	0.61
Gallbladder	1.2	0	3.4	0.69	0.50	0.72
Ileum	115.4	0	1.2	0.81	0.50	0.62
Jejunum	4.4	0	2.1	0.74	0.50	0.68
Kidney	12.4	2.3×10^{-2}	3.1	0.68	14.07	20.69
Liver	5.6	0	3.9	0.73	0.52	0.71
Lung	1.3	0	2.7	0.75	0.49	0.65
Lymph node	17.9	0	2.1	0.82	0.53	0.65
Myocardium	33.5	0	1.0	0.85	0.52	0.61
Ovary	0.8	0	4.4	0.83	0.50	0.60
Pancreas	44.8	0	1.1	0.78	0.53	0.68
Plasma	0.2	0	2.7	0.80	0.54	0.68
Skeletal muscle	24.1	0	1.2	0.85	0.61	0.72
Skin	244.7	0	1.0	0.69	0.38	0.55
Spleen	0.5	0	4.0	0.70	0.48	0.69
Tendon xanthomatous	1.8	0	66.6	0.03	0.03	0.93
Tendon unaffected	94.7	0	10.6	0.08	0.05	0.63
Thyroid	6.4	0	1.9	1.00	0.56	0.56
Uterus	1.2	0	1.8	0.82	0.54	0.66

* Concentration per gram wet weight of tissue. ‡ Specific activity dose-normalized to percent dose per gram cholesterol and percent dose per milligram squalene.

Table III. Plasma Cholesterol Specific Activity following Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol in Patient N.A.

Day	Cholesterol specific activity		
	^{3}H	^{14}C	Ratio (^{14}C/^{3}H)
	*% dose/g**	*% dose/g**	
1	3.44	1.39	0.404
4	2.64	1.12	0.424
8	1.71	0.88	0.515
11	1.29	0.73	0.566
15	0.96	0.58	0.604
18	0.80	0.54	0.675

* Specific activity dose-normalized to percent dose per gram cholesterol.

tissues take up circulating MVA and rapidly convert it to squalene that is slowly metabolized to cholesterol. The cholesterol specific activity data further support this concept in that the ratio of newly synthesized [^{14}C]cholesterol to intravenously administered [^{3}H]cholesterol is highest in the kidneys when compared with plasma or other body tissues (Table V), demonstrating that the [^{14}C]cholesterol in the kidneys did not arise from transport of the radiolabeled cholesterol from another site of synthesis. The mean ^{14}C/^{3}H isotope ratio in 12 tissues was 0.36±0.06 by day 15; in contrast, the kidney specific activity ratio was 1.11, a value that is three-fold higher than that found in other tissues. The calculated turnover of plasma cholesterol, whether [^{3}H]cholesterol or [^{14}C]cholesterol, gave a $t_{1/2}$ of ~6 d; the turnover of kidney [^{14}C]cholesterol was significantly slower with a $t_{1/2}$ of 14 d. Interestingly, kidney squalene exhibited a $t_{1/2}$ of 1.2 d, whereas no detectable radiolabeled squalene was found in the plasma at any time nor in other body tissues 15 d post infusion.

It is apparent from these data that the metabolism of circulating MVA through squalene to cholesterol occurs very rapidly in the majority of tissues, but not in the kidneys. The newly synthesized kidney squalene exhibits a slow turnover rate resulting in high specific activity cholesterol that then appears to equilibrate with plasma cholesterol.

Squalene and cholesterol synthesis from mevalonate in tissues of the rhesus monkey: 24-h study. Monkey no. 2 was sedated with ketamine, intravenously infused with *R,S*-[5-^{14}C]MVA and [1,2-^{3}H]cholesterol, and maintained under sedation for 8 h to permit serial biopsies of liver, kidney, and muscle tissues along with blood samples at 2, 4, 6, and 8 h post infusion. The animal was then returned to a metabolic cage for collection of feces and urine. 24 h post infusion the animal was killed for tissue sampling and measurement of squalene and cholesterol masses and specific activities.

Table VI presents the results of the serial analyses and demonstrates that circulating MVA was rapidly taken up by and converted to squalene in the kidney tissues; the dose-normalized specific activity of kidney squalene was 30-fold greater than that found in the liver. Interestingly, the liver squalene specific activity remained relatively constant over the 24-h period (0.97±0.10 percent dose/mg, n = 5), whereas plasma squalene exhibited a rapid turnover ($t_{1/2}$, ~1.2 h) and was completely absent by 24 h. These findings demonstrate the lack of equilibration between plasma and hepatic squalene; the two pools appear to exhibit strikingly different half-lives and synthesis rates. Muscle tissue takes up very little circulating MVA for metabolism to squalene as demonstrated by the low squalene specific activity at all time points tested.

The equilibration of newly synthesized [^{14}C]cholesterol and administered [^{3}H]cholesterol appears to be very rapid in liver and muscle tissues (^{14}C/^{3}H ratios of 0.38±0.04, n = 5, and 0.77±0.02, n = 5, respectively). In contrast, the ratio of specific activities in the kidneys is substantially higher; this, when combined with the elevated level of radiolabeled squalene, suggests a rapid and continued conversion of [^{14}C]squalene to [^{14}C]cholesterol in the kidneys over the 24-h period. The product of the conversion of squalene to cholesterol must equilibrate fairly rapidly with plasma cholesterol, since only 26% of the decrease in total [^{14}C]squalene radioactivity between 8 and 24 h is accounted for by a corresponding increase in total renal [^{14}C]cholesterol total radioactivity when calculated for the total mass of renal tissue (Table VII).

Table VIII presents the tissue analyses of squalene and cholesterol masses and specific activities at 24 h. Only kidney tissue contained appreciable amounts of radiolabeled squalene and a significantly different ^{14}C/^{3}H cholesterol isotope ratio (3.29 in renal tissue vs. 0.74±0.27 for 18 other tissues). The distributions of [^{3}H]cholesterol and ^{14}C-radioactivity are presented in Table IX: 93.1% of the total [^{3}H]cholesterol administered was recovered in the tissues, carcass, and feces; in contrast, ^{14}C-radioactivity was recovered in the urine (22.7%), and in the squalene/cholesterol fraction of the plasma, tissues, carcass, and feces (67.7%), totaling 85.7% of the total dose (some part of the 14.3% unaccounted for was presumably converted to $^{14}CO_2$ and nonsteroid isoprenoids). It should be noted that fecal steroids exhibited significantly different cholesterol isotope ratios with fecal neutral steroids having a ^{14}C/^{3}H ratio of 3.24 and acidic sterols having a ratio of 0.22; this suggests that newly synthesized cholesterol makes a major contribution to biliary cholesterol and fecal neutral steroids to a greater extent than to fecal bile acids (data not shown).

In vivo alterations in MVA metabolism in the kidneys of

Table IV. Tissue Squalene and Cholesterol Specific Activities in Rhesus Monkey No. 1 following Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Tissue	Time	Squalene specific activity	Cholesterol specific activity		
			^{3}H	^{14}C	Ratio (^{14}C/^{3}H)
	d	*% dose/mg*	*% dose/g**	*% dose/g**	
Plasma	2	0	98.97	41.05	0.41
	4	0	61.71	17.77	0.29
	8	0	35.11	14.44	0.41
	15	0	25.82	10.74	0.42
Kidney	2	60.22	15.31	45.70	2.98
	4	4.86	32.73	35.15	1.07
	8	0.84	21.74	30.16	1.39
	15	0.03	21.45	23.77	1.11

* Specific activity dose-normalized to percent dose per milligram squalene and percent dose per gram cholesterol.

Table V. Tissue Squalene and Cholesterol Mass and Specific Activity in Rhesus Monkey No. 1 15 d after Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Tissue	Squalene	Squalene	Cholesterol Mass	Cholesterol ^{3}H	Cholesterol ^{14}C	Ratio (^{14}C/^{3}H)
	*μg/g**	*% dose/mg‡*	*mg/g**	*% dose/g‡*	*% dose/g‡*	
Adipose	39.1	0	0.98	27.76	10.16	0.37
Adrenal	25.4	0	15.70	31.68	12.92	0.41
Aorta	3.9	0	1.07	26.43	5.50	0.21
Connective	18.0	0	0.51	26.85	8.43	0.31
Gallbladder	11.0	0	2.18	22.73	7.99	0.35
Kidney	13.1	0.03	2.23	21.45	23.77	1.11
Liver	7.5	0	2.18	19.15	8.47	0.44
Intestine						
Mucosa	4.8	0	1.40	15.82	5.36	0.34
Wall	11.9	0	1.69	18.69	6.81	0.36
Muscle	2.8	0	0.65	20.99	7.07	0.34
Ovary	4.1	0	4.92	22.59	8.68	0.38
Plasma	0.1	0	0.72	25.82	10.74	0.42
Skin	6.7	0	1.73	21.74	8.31	0.38

* Concentration per gram wet weight tissue. ‡ Specific activity dose-normalized to percent dose per milligram squalene and percent dose per gram cholesterol.

man. If the hypothesis that renal uptake and catabolism of circulating MVA accounts for the initial disequilibrium between exogenously administered [^{3}H]cholesterol and [^{14}C]cholesterol produced from [^{14}C]MVA, then the fractional conversion of MVA to cholesterol would be expected to be immediate in patients without kidneys. To test this possibility, two surgically anephric patients (V.F. and T.J.) were infused intravenously with [1,2-^{3}H]cholesterol and [5-^{14}C]MVA, and plasma samples were collected over the course of 21 d to determine the isotope ratio. An additional study was carried out in a male subject (A.A.) who had had one kidney removed surgically and demonstrated normal renal function in his remaining kidney. As shown in Fig. 1 *A*, the two anephric patients showed a plasma cholesterol isotope ratio that remained constant within 1 d after isotope infusion. The uninephric subject required 10 d to attain a constant ratio, approximately half the time required for control subjects with two functional kidneys (Fig. 1 *B*).

To further validate these findings, studies were carried out testing the effect of a preinfusion intravenous load of cold MVA (200 mg) to patients in the attempt to saturate renal MVA uptake. Similar studies by Hellstrom et al. (13) in the rat demonstrated that dilution of the radiolabeled MVA with cold MVA significantly reduced renal uptake of intravenously administered tracer MVA. We obtained similar findings in two patients with normal renal function (M.W. and B.K.): the plasma isotope ratio reached equilibrium within 10 d, as compared with 21 d, when cold MVA was infused 30 min before administration of the radiolabeled tracers for measurement of MVA fractional conversion (Table X). Infusion of the MVA mass also resulted in a 19% reduction in the calculated fractional conversion of radiolabeled MVA to cholesterol, consistent with a reduced uptake of MVA by the renal tissues; previous studies have demonstrated that the MVA fractional conversion measurement has a reproducibility of ±5% (8).

Table VI. Tissue Squalene and Cholesterol Specific Activity in Rhesus Monkey No. 2 following Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Tissue	Time	Squalene specific activity	Cholesterol specific activity ^{3}H	Cholesterol specific activity ^{14}C	Ratio (^{14}C/^{3}H)
	h	*% dose/mg*	*% dose/g**	*% dose/g**	
Liver	2	1.03	348.54	145.59	0.42
	4	0.95	320.22	122.17	0.38
	6	0.84	244.09	89.57	0.37
	8	1.11	122.89	39.80	0.32
	24	0.92	68.26	29.53	0.43
Plasma	2	1.02	40.33	49.84	1.24
	4	0.13	96.70	85.15	0.88
	6	0.06	87.06	59.87	0.69
	8	0.04	96.18	61.58	0.64
	24	0.0	167.69	93.03	0.55
Kidney	2	36.21	5.77	24.00	4.16
	4	28.54	10.78	29.29	2.72
	6	25.33	11.96	25.90	2.16
	8	30.36	6.70	18.34	2.74
	24	13.16	13.34	43.89	3.29
Muscle	2	0.02	10.75	8.36	0.77
	4	0.02	11.65	8.85	0.76
	6	0.02	14.69	11.62	0.79
	8	0.02	9.63	7.38	0.77
	24	0.02	4.37	3.23	0.74

* Specific activity dose-normalized to percent dose per milligram squalene and percent dose per gram cholesterol.

Table VII. Total Liver and Kidney [^{14}C]Squalene and [^{14}C]Cholesterol in Rhesus Monkey No. 2 following Intravenous Infusion with R,S-[5-^{14}C]MVA

Tissue	Time	[^{14}C]Radioactivity in: Squalene	Cholesterol
	h	*% dose/organ*	*% dose/organ*
Liver	2	1.1	30.2
	4	1.0	25.4
	6	0.9	18.6
	8	1.2	8.3
	24	1.0	6.1
Kidney	2	8.0	1.0
	4	6.3	1.2
	6	5.6	1.1
	8	6.7	0.8
	24	2.9	1.8

As shown in Table X, analysis of the initial rate of equilibration of the isotope ratio of newly synthesized and exogenous cholesterol (see Fig. 1 *B*) demonstrate that in control subjects the intercept on day 0 was 50% of the final isotope ratio, with a rate of change of 3.27%/d; in anephric patients, the intercept was ~100% with a slope of between 0.01–0.12%/d, and in the uninephric patient, values midway between the controls and the anephrics were obtained. In patients M.W. and B.K., given a preinfusion loading dose of cold MVA, the kinetics are similar to that observed in the uninephric subject, indicative of a reduced uptake of [^{14}C]MVA by the kidneys.

Measurement of renal uptake of plasma mevalonate. In an attempt to estimate the mass uptake of circulating MVA by the kidneys measurements of arterial-venous differences were carried out in patients undergoing surgery for carcinoma of the colon. While these data do not necessarily represent the normal in vivo situation (fasting, surgical trauma, unknown effects of anesthetics, etc.), they do provide some quantitative estimate of the ability of the kidneys to take up plasma MVA and, when combined with urinary output data, provide an estimate of MVA utilization via urinary excretion and catabolic processes. The arterial concentration of MVA in two such patients were 102 and 97 nM, and in renal vein plasmas were 48 and 71 nM, respectively. The renal uptake of MVA in these two subjects of 53 and 27% compare well with the data for arterial-venous differences found in the rat (2) and in the perfused rat kidney (16); these averaged ~34%, which can be compared with the mean of 40% found in these two subjects. Analysis of the plasma MVA concentrations in the two anephric patients (T.J. and V.F.) demonstrated a significant 3 to 10-fold increase (468 and 141 nM, respectively) as compared with a mean value in control subjects of 45 nM (3, 10); thus, patients who lack the ability to clear circulating MVA via the renal pathway have significantly higher plasma MVA levels.

Discussion

MVA plays a central role in overall polyisoprenoid biosynthesis as the product of the rate-limiting enzyme HMG-CoA reductase

Table VIII. Tissue Squalene and Cholesterol Mass and Specific Activity in Rhesus Monkey No. 2 at 24 h after Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Tissue	Squalene	Squalene	Cholesterol Mass	^{3}H	^{14}C	Ratio ($^{14}C/^{3}H$)
	*μg/g**	*% dose/mg‡*	*mg/g**	*% dose/g‡*	*% dose/g‡*	
Adipose	641.1	0.010	0.63	10.65	7.97	0.75
Adrenal	49.8	0.045	2.72	47.94	24.21	0.51
Aorta	5.2	0.047	0.70	25.83	14.41	0.56
Colon	12.2	0.034	2.14	3.57	3.08	0.86
Ileum	11.1	0.043	2.50	6.67	5.87	0.88
Jejunum	9.8	0.046	2.74	8.28	8.15	0.98
Kidney	10.6	13.163	1.97	13.34	43.89	3.29
Heart	24.6	0.049	1.08	15.49	8.74	0.56
Liver	10.3	0.917	2.01	68.26	29.53	0.43
Lungs	—	—	3.60	18.70	11.48	0.61
Muscle	21.4	0.019	0.48	4.37	3.23	0.74
Ovaries	2.0	0.147	3.36	4.86	3.73	0.77
Pancreas	12.2	0.161	1.25	11.65	9.52	0.82
Plasma	0.1	0.0	1.67	167.69	93.03	0.55
Skin	18.8	0.016	0.22	7.09	6.42	0.91
Spleen	1.5	0.196	3.67	116.86	24.31	0.21
Tendon						
Achilles	0.7	0.038	0.20	2.51	2.89	1.15
Psoas	6.6	0.026	0.32	4.19	5.61	1.34
Carcass	—	—	1.64	3.73	2.65	0.71

* Concentration per gram wet weight of tissue. ‡ Specific activity dose-normalized to per dose per gram cholesterol and per dose per milligram squalene.

Table IX. Distribution of Radiolabeled Squalene and Sterols in Rhesus Monkey No. 2 at 24 h after Intravenous Infusion of R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Tissues	Percent total dose recovered	
	[^{3}H]Cholesterol	[^{14}C]Squalene plus sterols
Plasma	41.2	22.8*
Liver	14.2	7.1
Kidney	0.6	4.7
Urine	0	22.7‡
Carcass plus feces	37.1	28.4
Total	93.1	85.7

* Body weight, 4.65 kg; blood volume, 75 ml/kg; and a 42% hematocrit.

‡ Urinary output value calculated for *R*-[5-^{14}C]MVA after subtraction of 50% of the total dose of *R,S*-[5-^{14}C]mevalonate infused from the total urinary ^{14}C-radioactivity, since the nonmetabolized *S*-form is quantitatively excreted in urine.

(1). The metabolism of circulating MVA has been investigated in a number of animal systems; its regulation is a complex interplay of dietary (2, 3), hormonal (17–21), and metabolic (2, 3, 22–24) processes. Plasma levels of MVA have been shown to be altered by diurnal patterns (2, 3, 24), dietary cholesterol (2, 3), fasting (2, 3), and drugs that induce (2, 3) or inhibit (10) HMG-CoA reductase in the rat and in man. The metabolism of circulating MVA to CO_2 by the trans-methyl-glutaconyl shunt (25) varies by sex, with females producing significantly more $^{14}CO_2$ from [5-^{14}C]MVA than males in the rat (17) and in humans (18).

Metabolism of circulating MVA in rhesus monkey and man. The data presented in the present study demonstrate that in humans and rhesus monkeys, as in the rat, the kidneys

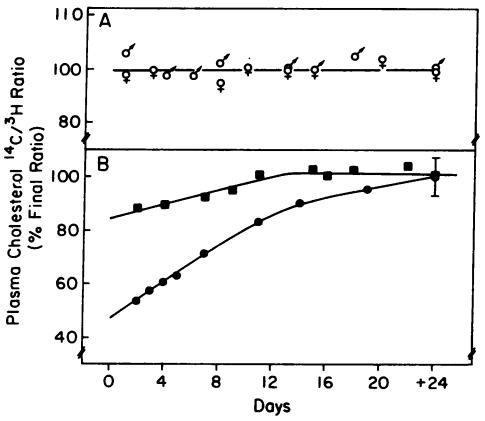

Figure 1. Plasma cholesterol isotope ratio kinetics in anephric, uninephric, and control patients following intravenous infusion with *R,S*-[5-^{14}C]MVA and [1,2-^{3}H]cholesterol. (*A*) Two anephric patients (V.F. and T.J.) were infused with radiolabeled MVA and cholesterol and the dose-normalized plasma cholesterol isotope ratio measured over the following 24 d. Data presented as percent of the final isotope ratio on day 24. (*B*) A uninephric patient, A.A. (■), and 15 control patients (●) were infused with radiolabeled MVA and cholesterol and the dose-normalized plasma cholesterol isotope ratio measured over the following 24 d. Data presented as percent of the final isotope ratio on day 24 or beyond.

Table X. Cholesterol Isotope Ratio Kinetics in 15 Control Studies and in Patients with Altered Renal Metabolism following Intravenous Infusion with R,S-[5-^{14}C]MVA and [1,2-^{3}H]Cholesterol

Patients	Isotope ratio kinetics	
	Intercept*	Slope
	day 0	*Δ ratio/d*
Controls (n = 15)‡	50±11	3.27±0.84
Anephric		
Patient V.F.	101	0.12
Patient T.J.	98	0.01
Uninephric		
Patient A.A.	85	1.24
Cold MVA dose		
Patient M.W.	83	1.22
Patient B.K.	74	1.48

* Intercept at day 0 calculated from initial slope of the isotope ratio curve (see Fig. 1 *B*).

‡ Control studies in patients with normal renal function previously reported (4, 8).

are a major site of circulating MVA metabolism. One form of this metabolism involves renal uptake of MVA and its conversion to squalene in the tissue; this squalene is then slowly converted to cholesterol that equilibrates with the plasma cholesterol pool. In the single study carried out in an autopsied human subject, the renal squalene pool was found to be significantly radiolabeled; the cholesterol pool in the kidneys had a dose-normalized specific activity almost 30-fold greater than any other tissue 18 d after intravenous infusion with radiolabeled MVA. As determined by the ratio of the dose-normalized cholesterol specific activities of administered and newly synthesized cholesterol, only the kidneys showed a disproportionate enrichment with the sterol synthesized from radiolabeled MVA.

In the monkey studies, the time-course of this renal uptake and metabolism of circulating MVA could be more clearly defined; a similar overall metabolic pattern was documented. Of all the tissues analyzed, only the kidneys retained a significant ratio of newly synthesized to administered cholesterol. In all the other tissues there was a rapid equilibration of the two isotopically labeled cholesterols such that the isotope ratios were almost identical, whether tested at 24 h or 15 d post infusion.

Comparison of MVA metabolism in different species. This study allows one to calculate total circulating MVA utilization in the rhesus monkey for comparison to published data collected in the rat and in man (Table XI). Assuming that the majority of the trans-glutaconate shunt occurs in the kidneys (26), one can calculate that as much as 42% of the metabolism of circulating MVA occurs in renal tissues in the monkey; similar calculations have been reported for the rat where 85% of the metabolism of circulating MVA occurs in the kidneys (14). In the human, urinary excretion of *R*-MVA and CO_2 production account for a total of 39–46% of its catabolism. Thus, renal metabolism of circulating MVA in man appears to be the major site of MVA catabolism via three pathways:

Table XI. In Vivo Metabolism of Intravenously Infused R,S-[5-^{14}C]MVA

Catabolism	Percent administered dose		
	Rat	Rhesus	Human
Urinary excretion	39	23	29
CO_2 production plus nonsteroid products	20	10	10–17
Sterol synthesis			
Kidney	26	9	14
Liver	4	30	40
Carcass	11	28	

urinary excretion, the trans-methyl-glutaconate shunt, and conversion to cholesterol via a squalene intermediate with a long $t_{1/2}$.

Radiolabeled MVA and in vivo kinetic studies. These data raise a number of questions regarding the suitability of radiolabeled MVA, administered intravenously, as a tracer for metabolic studies. One assumption inherent in long-term kinetic assays of sterol metabolism using MVA kinetics is the establishment of isotopic equilibrium between the plasma and tissue pools of the tracer; as previously reported, this equilibration is not obtained when MVA is given intravenously (10). In the present study we have demonstrated that this disequilibrium extends to intermediates derived from infused [^{14}C]MVA, newly synthesized squalene, and cholesterol. In our original studies of squalene kinetics using [5-^{14}C]MVA as tracer, we assumed that plasma and liver squalene were in isotopic equilibrium; however, as shown by the data in Table VI, it is obvious that, within the initial 24 h post infusion, hepatic and plasma squalene do not reach equilibrium and, when plasma squalene has been completely metabolized, significant amounts of radiolabeled squalene are still present in the liver. These findings indicate that even though there exists a quantitative relationship between plasma squalene kinetics and whole body cholesterol synthesis (4, 8), the theoretical basis upon which the method was proposed is incorrect.

This disequilibrium accounts for the delayed appearance of [^{14}C]cholesterol in the plasma following infusion of [^{14}C]MVA and [^{3}H]cholesterol. The data suggest that use of the plasma cholesterol isotope-ratio does not provide an accurate estimate of [^{14}C]MVA conversion to cholesterol, since the two radiolabeled cholesterols are kinetically distinct during the initial 21 d after infusion. The exogenously administered [^{3}H]cholesterol, given as a single bolus, is catabolized to bile acids and excreted as biliary cholesterol at the same rate as the newly synthesized cholesterol in the plasma. However, the input of [^{14}C]cholesterol from the kidneys provides a new source of radiolabeled cholesterol, and therefore, the apparent turnover rates of exogenous and newly synthesized cholesterol differ until such time that the input of [^{14}C]cholesterol from the kidneys becomes negligible. This contrast in input-output rates of the two cholesterol tracers may explain the findings of Kekki et al. (5) of significant synthesis of cholesterol in tissues thought to comprise pool two of total cholesterol metabolism following infusion of radiolabeled MVA and $^{3}H_2O$ in man. The apparent turnover of the labeled cholesterol from MVA would be expected to define kinetically the renal synthesis of cholesterol from the tracer; this process is significant in terms of radioactivity but not in terms of mass.

Quantitative significance of renal MVA metabolism. It has been hypothesized that impaired renal metabolism of MVA may play a significant role in the causation of the hypercholesterolemia prevalent in patients with renal diseases (27, 28), diabetes (19), thyroid dysfunction (20), and hypercholesterolemia (22). The quantitative significance of the renal metabolism of MVA must be considered not by the analysis of intravenously infused radiolabeled MVA but rather by the actual flux of total MVA through the plasma compartment to the kidneys. The rate of flux of newly synthesized MVA from sites of synthesis in the tissues to the plasma compartment can be determined from three sorts of data: urinary MVA output and renal clearance; plasma MVA turnover data and plasma concentration measurements; and steady state MVA kinetic measurements of plasma MVA input rates (10). Urinary output averages 2 μmol/d (9, 10), accounting for 30% of the total renal flux of MVA (9); thus, the kidneys are exposed to ~7 μmol MVA/d. Plasma MVA concentrations average ~50 nM with a $t_{1/2}$ of 40 min; this, in a 70-kg man, would represent a daily turnover of 3 μmol/d (2, 10). Steady-state isotope infusion studies of plasma MVA kinetics provided data indicating that the plasma compartment constitutes a defined pool of MVA metabolism and that MVA flux through the plasma compartment equaled 6 μmol/d (10). The composite data indicate that plasma MVA turnover could account for 3–7 μmol of MVA/d while total daily MVA production required to account only for daily cholesterol synthesis (and therefore a minimum estimate) is 12 mmol/d (11 mg cholesterol/kg per d × 70 kg × 1 mmol cholesterol/386.6 mg cholesterol × 6 mmol MVA/mmol cholesterol). The total MVA flux through the plasma compartment, and thus, through the kidneys, accounts for <0.1% of the total MVA production in man. A recent report by Weinstock et al. (29) arrives at the same conclusion from studies in the rat; the kidneys account for <0.1% of the metabolism of mevalonate in the body. While circulating MVA may play an important role in polyisoprenoid synthesis in the kidneys and possibly other peripheral tissues, it is apparent that any pathology that would alter renal metabolism of circulating MVA would have only a minor quantitative effect on whole body MVA metabolism or cholesterol synthesis.

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