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The Quantitative Role of the Kidneys in the *in Vivo* Metabolism of Mevalonate*

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The roles of the sterol and nonsterol pathways in the metabolism of circulating mevalonate have been estimated in the intact rat. On an average, the sterol pathway accounts for 74% of the mevalonate metabolized, while the nonsterol, or shunt, pathway is responsible for 26% of the mevalonate metabolized in the whole animal. The contribution of the kidneys to each of these processes was evaluated by two approaches. First, the localization of labeled sterols and sterol precursors derived from [^{14}C]mevalonate was determined in each of the major tissues of the body and, second, the effect of nephrectomy upon mevalonate metabolism by the sterol and shunt mechanisms was examined.

The results confirm our earlier conclusion that the kidneys represent the primary tissue site of conversion of circulating mevalonate to sterols and sterol precursors. In the present study, it was shown that by 6 h after administration of [^{14}C]mevalonate, the major end product of mevalonate metabolism in the kidneys is cholesterol and that, moreover, the kidneys are responsible for most of the cholesterol synthesized in the intact animal from injected mevalonate. Following nephrectomy, the extrarenal tissues can readily assume the dominant role normally played by the kidneys in synthesizing cholesterol and other sterols from circulating mevalonate.

The major observation of the present study is that the kidneys represent the primary site of mevalonate metabolism by the shunt pathway, in that nephrectomy results in approximately a 60% decrease in the mevalonate metabolized by the shunt pathway. These studies, therefore, reinforce and expand the evidence that the kidneys represent the most important single tissue site for the metabolism of circulating mevalonate.

It is well established that in almost all animal species the liver represents the major tissue site of cholesterol synthesis from two-carbon units (1-3). Hepatic cholesterogenesis, moreover, is known to be controlled by a sensitive negative feedback system (4-7) that operates primarily at the site of synthesis of the cholesterol precursor, mevalonic acid (8-11). This feedback regulation of mevalonate synthesis has been shown to be defective or completely deleted in at least two pathologic conditions, familial hypercholesterolemia (12, 13) and malignancy (14-16) as well as premalignancy (9, 17) in the intact animal. Since such derangements of the cholesterol feedback system would result in the abnormal regulation of mevalonic acid synthesis (12, 18, 19), studies were undertaken to determine the metabolic fate of the mevalonic acid that is released into the circulation under normal and pathologic conditions. To date, such studies have unexpectedly demonstrated that the major tissue site of the metabolism of circulating mevalonate is not the liver, but the kidneys (20, 21). In these earlier studies the kidneys, in fact, were found to convert circulating mevalonate to sterols and sterol precursors at rates at least four times that of the liver (20).

With the observation of Edmond and Popják (22) and of Fogelman *et al.* (23) that injected mevalonate can be metabolized in the intact organism by a pathway that by-passes sterol synthesis, studies were initiated to determine whether the kidneys also play the dominant role in metabolizing mevalonate via this nonsterol, or "shunt," pathway. The results of such studies demonstrated that, by *in vitro* assay, the kidney is by far the most active site of mevalonate metabolism by this newly described shunt pathway (24); renal tissue, in fact, oxidized mevalonate to CO_2 at rates that averaged over 20 times that of any other tissue examined.

In the present study, the quantitative role of the kidneys in metabolizing mevalonate by both the sterol and nonsterol pathways was evaluated *in vivo* by comparing the fate of circulating mevalonate in normal and nephrectomized animals. These results demonstrated that under *in vivo* conditions the kidneys are the major tissue site for the metabolism of circulating mevalonate by both the sterol and nonsterol pathways.

EXPERIMENTAL PROCEDURES

Animals and Procedures—Male Sprague-Dawley rats weighing between 250 and 400 g were maintained on Purina rat chow and water *ad libitum* until 24 h before the experiment, after which food was withheld. The animals were anesthetized with diethyl ether and the kidneys were removed from the experimental rats through a midline abdominal incision. Laparotomy and manipulation of the kidneys were carried out in the control rats. Immediately after surgery, the rats were injected, through the tail veins, with potassium [^{14}C]mevalonate dissolved in 0.4 ml of 0.9% NaCl solution. Unless otherwise noted, 500 nmol of (*R,S*)mevalonate were administered to each rat. They were then placed in 2-liter wide mouth Erlenmeyer flasks fitted with two-hole stoppers. Air was drawn through the bottles at a rate of approximately 4 liters/h, and expired $^{14}CO_2$ was continuously collected in gas-washing bottles containing

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180 ml of 1 N NaOH. To ensure complete trapping of the CO_2, a second gas-washing bottle also containing 1 N NaOH was connected in tandem with the primary CO_2 collecting bottle. At the times noted, 0.2-ml samples of the NaOH were added to 10 ml of scintillation solution, and ^{14}C was determined on a Beckman LS-230. After 6 h the rats were killed, and the organs and carcass were saponified separately and analyzed for ^{14}C-lipids as described below.

Materials—(*R,S*)-[5-^{14}C]Mevalonic acid (13 mCi/mmol) was purchased from Schwarz/Mann, the (*R,S*)-[2-^{14}C]mevalonolactone (18 mCi/mmol) was obtained from Amersham/Searle, and the unlabeled (*R,S*)-mevalonolactone from Sigma Chemical Co. All mevalonic acid was converted to its potassium salt prior to injection. The [1,2-^{3}H]cholesterol (31 Ci/mmol) used as an internal standard was purchased from New England Nuclear. The thin layer Polygram Sil G plates were purchased from Brinkmann Instruments. Radioautography was carried out on Kodak RP-14 x-ray film. The counting solution used for the $^{14}CO_2$ samples was prepared as follows: 300 ml of Beckman Bio-Solv III, 1000 ml of Packard scintillation grade toluene, 100 ml of glass-distilled water, and 6.0 g of 2,5-diphenyloxazole (Amersham/Searle). The thin layer strips were counted in 10 ml of the following scintillation fluid: 0.3 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (Amersham/Searle), 6.0 g of 2,5-diphenyloxazole, 133 ml of ethyl acetate, and 1867 ml of Packard scintillation grade toluene.

Analytical Methods—After the addition of internal standard of [^{3}H]cholesterol, the various tissues were separately saponified. After refluxing overnight in alcoholic KOH (90% wet weight approximately 10 ml/g of tissue) in Erlenmeyer flasks, the nonsaponifiable material was extracted once with 50 ml of petroleum ether. The petroleum ether extract was dried, dissolved in 1 ml of chloroform, and 250 μl were applied to the thin layer chromatographic plates. The plates were developed sequentially in ethyl acetate: benzene, 1:5, for 50 min, followed by ethyl acetate:benzene, 1:20, for 2 h. They were then radioautographed for 3 to 5 days, and the areas of the plates corresponding to standards of cholesterol, lanosterol, and squalene were cut out and placed in vials containing 10 ml of counting solution. The gain and discrimination window settings of the scintillation counter were adjusted so that less than 0.2% of the ^{3}H counts were read in the ^{14}C window and approximately 15% of the ^{14}C counts were recorded in the ^{3}H window. The amount of ^{3}H-labeled cholesterol added as internal standard was adjusted so that ^{3}H counts were approximately five times greater than the ^{14}C counts. Calculations were corrected for spillover of ^{3}H and ^{14}C and for background. The calculated recovery of the cholesterol averaged approximately 50%. Gas-liquid chromatography was carried out in a Varian model 2100, using a 5½-foot glass column packed with 3% QF-1 stationary phase on Gas-chrom Q (100 to 120 mesh). Gas flow was 19 ml/min, temperature 230°. Under these conditions cholesterol has a retention time of 5 min. The ^{14}C-labeled sterols were collected using a stream splitter, and the ^{14}C was determined on a Packard Tri-Carb 526.

Rationale of Evaluating Shunt Pathway of Mevalonate Metabolism—As described in detail in a previous publication (24), the mevalonate shunt pathway can be estimated by determining the $^{14}CO_2$ produced from either [2-^{14}C]mevalonate or, as first introduced by Fogelman *et al.* (23), from [5-^{14}C]mevalonate. In the case of [2-^{14}C]mevalonate, 1 molecule of $^{14}CO_2$ is released during the demethylation of lanosterol for every 5 atoms of ^{14}C incorporated into cholesterol (25). Accordingly, the calculation of [2-^{14}C]mevalonate metabolized to $^{14}CO_2$ via the shunt pathway requires the subtraction of the [^{14}C]cholesterol ÷ 5 from the total $^{14}CO_2$. Similarly, an estimate of the total [2-^{14}C]mevalonate utilizing the sterol pathway necessitates adding the same factor to the total recovered ^{14}C-sterols and ^{14}C-sterol precursors. Since [5-^{14}C]mevalonate is converted to $^{14}CO_2$ only by the shunt pathway, no such correction is needed when [5-^{14}C]mevalonate is used as the substrate.

(*R,S*)-[^{14}C]Mevalonate was employed throughout this study. Since only the (*R*) isomer or mevalonate is metabolized not only for sterol synthesis (25) but also by the shunt pathway (23), all calculations in this study assume that only one-half of the administered mevalonate was available to be metabolized.

RESULTS

Tissue Distribution of Mevalonate Metabolites—The tissue localization of ^{14}C-sterols and of ^{14}C-sterol precursors 6 h after the intravenous administration of [^{14}C]mevalonate to normal, intact rats is summarized in Fig. 1 (*open bars*). As the results

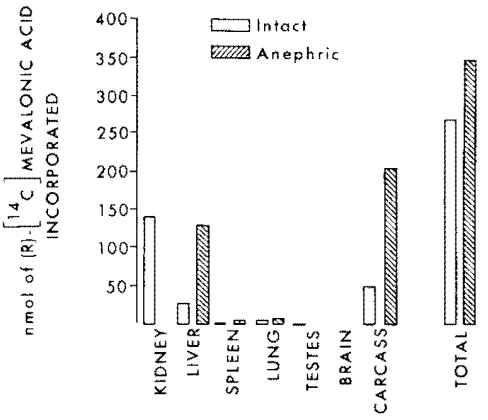

FIG. 1. Recovery of intravenously administered (*R*)-[^{14}C]mevalonate in sterols and sterol precursors in various tissues of intact rats (*open bars*) and anephric rats (*hatched bars*). For details see Table I.

obtained with [2-^{14}C]mevalonate and [5-^{14}C]mevalonate were comparable, the combined data are presented. In these five experiments, between 47% and 70% (average 61%) of the mevalonate metabolites found within the body was recovered in the kidneys. Moreover, these data demonstrate that the kidneys accumulate and metabolize mevalonate at a rate five times that of the liver. In fact, during the 6 h of the study, the two kidneys metabolized more of the circulating mevalonate than did all other tissues of the body combined.

Mevalonate Metabolites in Intact Animal—We have previously shown that the conversion of mevalonate to saponifiable material represents only a minor route of mevalonate metabolism (20); therefore, no attempt was made in the present study to analyze the acidic end products of mevalonic acid. The distribution of ^{14}C among the nonsaponifiable metabolites of intravenously administered [^{14}C]mevalonate, in the five experiments with [2-^{14}C]mevalonate or [5-^{14}C]mevalonate, is presented in Table I. [^{14}C]Lanosterol and [^{14}C]squalene could be identified in several tissues of the intact animal but most notably, in the kidneys and the carcass. On an average, 9% of the nonsaponifiable material in the kidneys was accounted for as lanosterol and 15% as squalene. However, at the 6-h interval studied, the major end product of mevalonate metabolism in all tissues studied was a 27-carbon sterol which, by thin layer chromatography, corresponded to cholesterol. Although in liver, as expected, cholesterol accounted for over 80% of the total labeled nonsaponifiable material recovered, the kidneys, surprisingly, also readily converted mevalonate to cholesterol. On an average, 76% of the ^{14}C in the nonsaponifiable material in the kidneys was recovered in the cholesterol band on thin layer chromatography. The identification of the [^{14}C]27-carbon sterol as cholesterol was confirmed by gas-liquid chromatography. As shown in Fig. 2, over 98% of the ^{14}C recovered in the 27-carbon band by thin layer chromatography was present in the cholesterol peak on the gas-liquid chromatogram (see "Experimental Procedures"). Measurement of the area under the sterol peaks in Fig. 2 also demonstrated that over 99% of the sterols present in the 27-carbon band on the thin layer plate was cholesterol. As will be emphasized below, of all organs studied, the kidneys were also found to be the most important single tissue site of cholesterol synthesis from circulating mevalonate.

Mevalonate Metabolism by Mevalonate Shunt and Sterol Pathways—Typical rates of $^{14}CO_2$ expiration for 6 h after the

TABLE I

In vivo metabolism of [^{14}C]mevalonate in intact and nephrectomized rats 6 h after intravenous administration of mevalonate

	Substrate: [2-^{14}C]Mevalonate[a]						Substrate: [5-^{14}C]Mevalonate[b]						Summary					
	Intact			Anephric			Intact			Anephric			Intact			Anephric		
	27-C	Lanosterol	Squalene	27-C	Lanosterol	Squalene	27-C	Lanosterol	Squalene	27-C	Lanosterol	Squalene	27-C	Lanosterol	Squalene	Total nonsaponifiable	27-C	Lanosterol	Squalene	Total nonsaponifiable
	nmol			*nmol*			*nmol*			*nmol*			*nmol*				*nmol*			
Kidney	127 ± 40	23 ± 12	27 ± 14				61 ± 7	5 ± 1	14 ± 0				101	16	22	139				
Liver	21 ± 5	1 ± 1	2 ± 2	83 ± 32	3 ± 1	14 ± 12	12 ± 16	2 ± 1	6 ± 3	142 ± 6	4 ± 0	26 ± 11	21	1	4	26	107	3	19	129
Spleen	1 ± 1	0	0	3 ± 1	0	0	1 ± 0.1	0	0	8 ± 0	0.1 ± 0.1	0	1	0	0	1	4	0	0	4
Lung	3 ± 1	0	0	8 ± 0	0	0	2 ± 0.3	0	0	1 ± 0	0	0	2	0	0	2	8	0	0	8
Testes	1 ± 0.4	0	0	1 ± 0	0	0	1 ± 0.3	0	0	0.8 ± 0	0	0	0.6	0.1	0.1	0.8	0.9	0	0	0
Brain	1 ± 0.3	0	0	0.8 ± 0.3	0	0.1 ± 0.1	0	0	0	0.4 ± 0	0	0	0.5	0	0	0.5	0.6	0	0	0.6
Carcass	42 ± 6	2 ± 1	6 ± 9	158 ± 35	7 ± 3	26 ± 37	28 ± 0.3	3 ± 1	19 ± 4	136 ± 0	8 ± 0	81 ± 13	36	2	11	49	149	8	48	205
Total (whole animal)	196 ± 44	26 ± 12	35 ± 17	254 ± 64	30 ± 31	39 ± 50	112 ± 1	10 ± 2	39 ± 1	291 ± 6	12 ± 0	107 ± 1	162	19	37	218	269	11	67	347
Total $^{14}CO_2$ expired	125 ± 11			91 ± 6			70 ± 3			53 ± 27			79				38			
Total $^{14}CO_2$ (shunt pathway)	85 ± 13			41 ± 8			70 ± 3			53 ± 27										

[a] Average of three experiments.
[b] Average of two experiments.

intravenous injection of [5-^{14}C]mevalonate to intact rats are presented by the *upper two curves* of Fig. 3. After a brief lag, $^{14}CO_2$ production from the [5-^{14}C]mevalonate increased rapidly, reaching a maximum rate at approximately 60 min and decreasing to less than 10% of this rate by the 6th h of the study. If the $^{14}CO_2$ produced from [^{14}C]mevalonate is assumed to follow first order kinetics, approximately 90% of the $^{14}CO_2$ resulting from [^{14}C]mevalonate oxidation was expired during this 6-h period. The shape of these curves and the rapid oxidation of [5-^{14}C]mevalonate to $^{14}CO_2$ in the intact rat fully confirm the similar findings of Fogelman *et al.* (23).

The total $^{14}CO_2$ produced during the three studies employing [2-^{14}C]mevalonate and during the two experiments utilizing [5-^{14}C]mevalonate is presented in Table I. After correcting for the $^{14}CO_2$ produced from [2-^{14}C]mevalonate by the sterol pathway, the total nanomoles of [^{14}C]mevalonate oxidized to $^{14}CO_2$

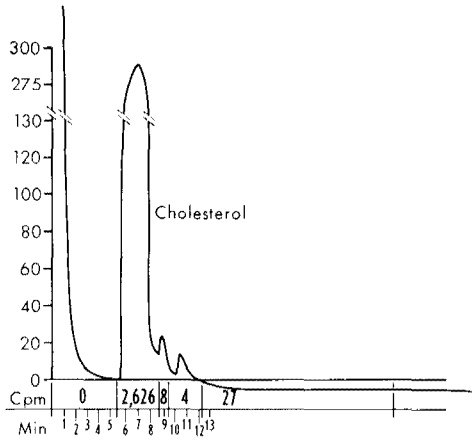

FIG. 2. Gas-liquid chromatographic separation of renal 27-carbon sterols 6 h after intravenous administration of [^{14}C]mevalonate. The major peak corresponding to a retention time of 5.5 min corresponds to that of a cholesterol standard. For details see "Experimental Procedures" and "Results."

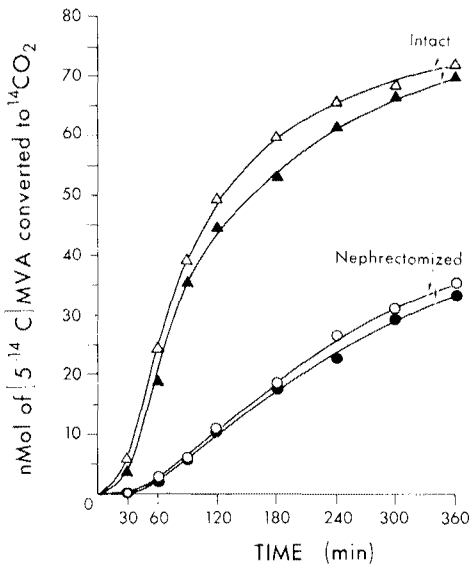

FIG. 3. Oxidation of [5-^{14}C]mevalonate (*MVA*) to $^{14}CO_2$ by intact and nephrectomized adult rats. The *triangles* represent the results in two intact rats and the *circles*, the results in two nephrectomized animals. See "Experimental Procedures" for details.

by the shunt pathway were calculated. At the 6-h interval studied, some of the ^{14}C derived from the administered [2-^{14}C]mevalonate and [5-^{14}C]mevalonate was no doubt trapped in compounds that had not been oxidized to $^{14}CO_2$. The resulting estimates of mevalonate shunt activity, presented in Table I, therefore represent minimum figures.

Since the conversion of mevalonate to sterol and sterol precursors was also determined (Table I), it was possible in the present studies to evaluate both the absolute and the relative roles of the shunt and sterol pathways of mevalonate metabolism in the intact animal. The quantitative roles of these two pathways for the five experiments are summarized in Table II. The absolute amounts of [^{14}C]mevalonate metabolized by the sterol and shunt pathways are given in Columns D and G of Table II. As indicated in Columns J and K of Table II, between 69 and 82% (average 74%) of the mevalonate metabolized by the intact rat utilized the sterol pathway, whereas 18 to 31% of the injected mevalonate was metabolized by the shunt pathway.

Role of Kidneys in Mevalonate Metabolism by Whole Animal—The role of the kidneys in mevalonate metabolism by the whole animal was evaluated by two approaches. First, renal metabolism of mevalonate by the sterol pathway was quantified by directly determining the amount of circulating [^{14}C]mevalonate incorporated into sterols and sterol precursors by both the kidneys and the remainder of the body. Second, the roles played by the kidney in circulating mevalonate metabolism by both sterol and shunt pathways were estimated by determining the effect of nephrectomy on the rat's ability to metabolize mevalonate through each pathway. For ease of comparison, the results of both approaches for the five experiments are presented in Table II.

The contribution of the kidneys to the overall metabolism of circulating mevalonate in the intact rat is indicated in Columns A, B, and C of Table II. As calculated in Column C, between 59 and 68% (average of 63%) of the mevalonate metabolized in the body (Column A) is carried out by the kidneys. Calculation of the renal contribution to the metabolism of mevalonate by the sterol pathway (Columns D, E, and F) again demonstrated that the kidneys are responsible for approximately two-thirds (53 to 70%) of the circulating mevalonate metabolized by this pathway.

Effect of Nephrectomy on Sterol Pathway of Mevalonate Metabolism—As is also documented by the data in Table II and summarized in Fig. 1 (*hatched bars*), nephrectomy had marked qualitative and quantitative effects upon the metabolism of plasma mevalonate. Despite the removal of the major tissue site of metabolism of circulating mevalonate, the rate of sterol synthesis from mevalonate was not depressed. In fact, in Experiments 1, 3, 4, and 5, a significantly greater amount of the injected mevalonate was metabolized by the sterol pathway in the nephrectomized animal than in the intact animal (Table II, Column D; Fig. 1). In three of the five experiments, the total recovered mevalonate metabolites were significantly increased following nephrectomy. The effect of nephrectomy upon mevalonate metabolism could be more accurately estimated by comparing the percentage of retained (*R*)-mevalonate metabolites utilizing the sterol pathway in the intact animal with that in the nephrectomized rat. As shown in Column L of Table II, when corrected for such differences in the absolute amount of retained mevalonate metabolized, the calculated mevalonate utilization via the sterol pathway was increased by an average of 22% following nephrectomy.

This paradoxical result is partially explained by the fact that in each of the five experiments the hepatic metabolism of mevalonate increased markedly after removal of the kidneys. Nephrectomy caused the liver to increase its rate of mevalonate metabolism via the sterol pathway by an average of 5-fold, *i.e.* from 26 nmol in the intact rat to 129 nmol in the anephric animal. Moreover, a comparable increase in mevalonate metabolism by the carcass of the nephrectomized rat was also observed, *i.e.* 49 nmol in the intact animal, increasing to 205

TABLE II

Role of kidney in mevalonate metabolism by sterol and shunt pathways

Observed during 6 h after intravenous administration of [2-^{14}C]mevalonate or [5-^{14}C]mevalonate.

Renal status	Total metabolites of (*R*)-[^{14}C]mevalonate recovered in			Total body and renal metabolism of mevalonate by						Per cent of total mevalonate metabolites derived from		Effect of nephrectomy on mevalonate metabolism by	
				Sterol pathway			Shunt pathway			Sterol pathway	Shunt pathway	Sterol pathway	Shunt pathway
	A. Total body	B. Kidney	C. Per cent of total metabolized by kidney = (B/A·100)	D. Total body	E. Kidney	F. Per cent of total metabolized by kidney = (E/D·100)	G. Total body	H. Kidney	I. Per cent of total metabolized by kidney = (H/G·100)	J. = (D/A·100)	K. = (G/A·100)	L. Per cent change in J caused by nephrectomy	M. Per cent change in K caused by nephrectomy
	nmol	*nmol*	%	*nmol*	*nmol*	%	*nmol*	*nmol*	%	%	%	%	%
Experiment 1													
Intact	374	238	64	274	182	66	100	56	56	73	27	+21	−56
Anephric	366			322			44			88	12		
Experiment 2													
Intact	326	196	60	250	176	70	74	26	35	77	23	+10	−35
Anephric	298			252			46			85	15		
Experiment 3													
Intact	450	304	68	368	250	68	82	54	66	82	18	+15	−67
Anephric	524			492			32			94	6		
Experiment 4													
Intact	232	148	64	164	100	61	68	48	71	71	29	+30	−72
Anephric	452			416			36			92	8		
Experiment 5													
Intact	230	136	59	158	84	53	72	52	72	69	31	+33	−74
Anephric	440			406			34			92	8		
Average													
Intact	322 ± 94	196 ± 77	63	242 ± 87	158 ± 67	64	79 ± 13	47 ± 12	60	74 ± 5	26 ± 5	+22	−62
Anephric	416 ± 87			378 ± 93			38 ± 6			90 ± 4	10 ± 4		

nmol in the nephrectomized rat. The liver and the carcass, then, responded to nephrectomy by increasing their contribution to the sterol pathway of mevalonate metabolism by a total of 259 nmol, an increment which completely compensated for the loss of the 158 nmol of mevalonate (Table II, Column E) converted to sterol and sterol precursors by the kidneys of the intact rat.

Effect of Nephrectomy on Shunt Pathway of Mevalonate Metabolism—The role of the kidney in mevalonate metabolism by the shunt pathway is indicated by the data in Column H of Table II. These figures were derived from the total $^{14}CO_2$ produced from [2-^{14}C]mevalonate or [5-^{14}C]mevalonate by the shunt pathway (Column G) and the observed decrease in $^{14}CO_2$ by this pathway following nephrectomy. As shown in Column I, by this calculation the kidneys were responsible for between 35 and 72% (average 60%) of the metabolism of circulating mevalonate by the shunt pathway.

In contrast to its effect of increasing mevalonate metabolism by the sterol pathway, in each of the five experiments nephrectomy caused a marked and consistent decrease in mevalonate metabolism by the shunt pathway (Fig. 3; Table II, Column G). On an average, the nephrectomized animal oxidized mevalonate to $^{14}CO_2$ by the shunt pathway at a rate that was only 48% (38 nmol × 100/79 nmol) of that of the intact animal. As also indicated by the data in Fig. 3, the decrease in $^{14}CO_2$ production resulting from nephrectomy persisted throughout the 6 h of the experiment. By correcting for retained mevalonate metabolites (Column M, Table II), we found that, on an average, nephrectomy caused a 62% reduction in the metabolism of mevalonate by the shunt pathway.

Effect of Mevalonate Concentration on Mevalonate Metabolism—In the experiments thus far described, 500 nmol of (*R*)-[2-^{14}C]mevalonate or (*R*)-[5-^{14}C]mevalonate were administered to the experimental animals. To determine whether lower or higher levels of mevalonate would influence the relative importance of the two routes of mevalonate metabolism, 69, 500, or 3850 nmol of (*R*)-[2-^{14}C]mevalonate were administered to intact rats and the total mevalonate metabolized over the sterol and shunt pathways was determined. The results in Table III indicate that a 55-fold increase in injected mevalonate resulted in a 38-fold increase in mevalonate metabolism over the sterol pathway. At the highest concentration, the amount of [^{14}C]mevalonate utilizing the sterol pathway increased from 46 to 1730 nmol. The activity of the shunt pathway, on the other hand, was enhanced to a relatively greater extent, *i.e.* from 7.2 to 648 nmol, a 90-fold increase.

When exposed to high levels of circulating mevalonate, both pathways of mevalonate metabolism can obviously, therefore, greatly expand their capacities to metabolize mevalonate. However, the dose-response of the shunt pathway is proportionately greater than that of the sterol pathway.

Mevalonate Metabolism in Infant Rats—Studies were next carried out to determine, first, whether mevalonate metabolism in 13-day-old animals followed the pattern seen in the adult rat and, second, whether the role of the kidney in the pathways of mevalonate metabolism in such newborn rats was similar to that in the adult animal. The experimental design was identical with that employed in the adult rats with the following exceptions: (*a*) groups of four normal and four nephrectomized animals were placed in two separate Erlenmeyer flasks, (*b*) the experiment was carried out for 3 instead of 6 h, (*c*) the [5-^{14}C]mevalonate was injected subcutaneously rather than intravenously, and (*d*) each rat received 1.0 ml of saline subcutaneously to prevent dehydration during the experiment.

TABLE III

Effect of mevalonate concentration on pathways of mevalonate metabolism

Injected (*R*)-[^{14}C]mevalonate	(*R*)-[^{14}C]Mevalonate metabolized by	
	Sterol pathway	Shunt pathway
nmol	*nmol*	*nmol*
69	46	7.2
500	251	75
3850	1730	648

The results shown in Table IV demonstrated that, as was found in the adult animal, the kidneys represent the major site of sterol synthesis from mevalonate in the intact infant rat. Moreover, the increase in sterol synthesis by the nonrenal tissues, which followed nephrectomy in the adult animal, was again observed in the infant rat; as in the adult, the liver became the major site of sterol synthesis in the anephric newborn animal.

The mevalonate shunt was found to be responsible for 14% of the total mevalonate metabolized in the infant rat. The effect of nephrectomy upon the mevalonate shunt pathway is demonstrated in Fig. 4 and Table IV. Removing the kidneys resulted in a 60% decrease in the oxidation of mevalonate via the shunt pathway.

Finally, studies were carried out to establish whether, under *in vitro* conditions, the kidneys of the newborn rat are the primary site of mevalonate shunt activity. Slices were prepared from various tissues of the 13-day-old rat and incubated with [5-^{14}C]mevalonate. The shunt and sterol pathways were

TABLE IV

Mevalonate metabolism in newborn rats in vivo

Organ	(*R*)-[^{14}C]Mevalonate converted to total nonsaponifiable lipids	
	Intact	Anephric
	nmol/3 h	*nmol/3 h*
Kidney	161	
Liver	39	109
Spleen	3	3
Lung	8	11
Intestine	10	59
Brain	1	2
Skin	60	57
Carcass	91	105
Total	373	345
	CO_2	
	52	21

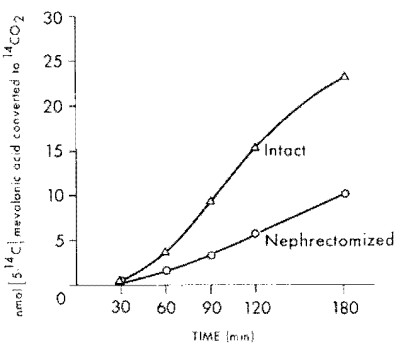

FIG. 4. Oxidation of [5-^{14}C]mevalonate to $^{14}CO_2$ by intact and nephrectomized 13-day-old rats.

assessed as previously described (24). As the results in Table V indicate, the kidney of the newborn rat is overwhelmingly the major site of mevalonate metabolism by the shunt pathway. As was the case in the adult animal, when studied *in vitro*, kidney slices converted mevalonate to sterols at rates comparable to that of liver.

These studies, therefore, indicate that, under both *in vivo* and *in vitro* conditions, the metabolism of mevalonate in the young rat is identical with that in the adult animal.

DISCUSSION

The findings of the present study serve to reinforce and extend our previous conclusion that the kidneys represent the major tissue site of metabolism of circulating mevalonic acid. Earlier studies demonstrated that in the intact animal, examined at 0.5 to 2 h after administration, approximately two-thirds of the injected mevalonate is taken up and metabolized by the kidneys (20).

We have, more recently, presented *in vitro* evidence (24) that the kidneys also play the primary role in the metabolism of mevalonate by the nonsterol, or shunt, pathway originally described in the intact animal by Edmond and Popják (22). A comparison of the ability of various tissue slices to oxidize [^{14}C]mevalonate to $^{14}CO_2$ demonstrated that, per weight of tissue, the kidneys are, on an average, over 20 times more active in metabolizing mevalonate by the nonsterol, or shunt, pathway than any other tissue of the body. It was observed in these earlier experiments, however, that in many of the extrarenal tissues examined, small but significant amounts of mevalonate were oxidized over the shunt pathway. The possibility remained, therefore, that in the intact animal these extrarenal tissues—which, of course, have a far greater total organ weight than do the kidneys—might, in the aggregate, play a significant role in the nonsterol pathway of mevalonate metabolism.

Moreover, since previous studies had not quantified the two known pathways of mevalonate metabolism in the intact animal, the overall importance of these two pathways of disposing of circulating mevalonate in the whole animal remained unknown. The present studies, therefore, were designed, first, to evaluate the relative roles of the sterol and shunt pathways of mevalonate metabolism in the intact animal and, second, to determine the role of the kidneys in the metabolism of circulating mevalonate by each of these metabolic routes.

A comparison of [^{14}C]mevalonate metabolites produced via the shunt and sterol pathways demonstrates (Table II) that the shunt pathway accounts for an average of 26% of the mevalonate metabolized in the intact animal, while the sterol pathway is responsible for 74% of the metabolized mevalonate. These results, therefore, clearly indicate that the shunt pathway plays a significant role in the metabolism of circulating mevalonate in the intact rat.

TABLE V

Metabolism of [5-^{14}C]mevalonate in tissue slices from infant rats

Organ	Mevalonate metabolism by	
	Sterol pathway	Shunt pathway
	nmol/g tissue/h	
Kidney	18.5	3.9
Liver	7.3	0.4
Lung	0.3	0.5
Spleen	0.1	0.5
Ileum	0.1	0.2
Brain	0.8	0.3
Skin	0.4	0.2

The results shown in Tables I and II fully confirm our earlier conclusions that the kidneys represent the primary tissue site of sterol production from circulating mevalonate. The role of the kidneys in the synthesis of cholesterol itself was, however, surprising. In a previous study (20) it was shown that 2 h after injection of mevalonate, the kidneys converted mevalonate to squalene and lanosterol and only to a lesser extent, to cholesterol. The present study, however, has demonstrated that, with time, the kidneys are readily capable of carrying the process of cholesterol synthesis to completion, in that at 6 h approximately 75% of the injected mevalonate had been incorporated into cholesterol with only 25% remaining in cholesterol precursors. More important, these data demonstrate that the kidneys represent the primary tissue site of cholesterol synthesis from circulating mevalonic acid, *i.e.* the kidneys actually accounted for an average of 60% of the total cholesterol synthesized by the body from circulating mevalonate.

The question might be raised as to whether the high levels of [^{14}C]cholesterol found in the kidneys after the injection of [^{14}C]mevalonate might reflect redistribution to the kidneys of cholesterol synthesized in the liver and released into the circulation, rather than true renal cholesterogenesis. This possibility seems highly unlikely for at least two reasons: first, our earlier studies (21, 24) have shown that renal tissue, studied *in vitro*, is capable of synthesizing sterols at least as rapidly as does liver, and second, it can be directly shown that during a 3-h period [^{14}C]cholesterol injected into the circulation is predominantly taken up by the liver (62%) and only a minute amount (less than 0.7%) of the labeled sterol is found in the kidneys.[1]

Removal of the kidneys was found to have strikingly different effects upon the metabolism of mevalonate by the sterol and the nonsterol pathways. Although in the intact animal the kidneys clearly play the major role in metabolizing circulating mevalonate by the sterol pathway, the data in Fig. 1 and Tables I and II show that, following the removal of the kidneys, the liver, and to a lesser extent the other nonrenal tissues, can very adequately assume the role in cholesterogenesis normally played by the kidneys. Thus, despite the dominant role played by the kidney in mevalonate metabolism in the intact animal, the removal of the kidneys did not result in a decrease in the overall metabolism of mevalonate to sterols and sterol precursors. In fact, the anephric rat actually metabolized more mevalonate to nonsaponifiable material than did the intact animal. A very similar increase in the mevalonate incorporated into nonsaponifiable lipids, following nephrectomy, has been reported by Cuzzopoli *et al.* (28).

At least three factors may be cited to account for this ability of extrarenal tissue to compensate for the loss of renal cholesterogenesis in the nephrectomized animal. First, nephrectomy undoubtedly results in a redistribution to extrarenal tissues of the 25% of the cardiac output that normally supplies the kidneys. Second, the circulating mevalonate, which in the intact animal is rapidly taken up and metabolized by the kidney through both the sterol and shunt pathways, will, in the nephrectomized animal, be available to other sterol-producing tissues. Third, some mevalonate is no doubt normally excreted in the urine, and this urinary loss of mevalonate is obviously prevented by nephrectomy. Due to these factors the extrarenal tissues of the nephrectomized animal would be

[1] M. M. Howton, M. H. Wiley, and M. D. Siperstein, unpublished results.

exposed to higher levels of [^{14}C]mevalonate than would those of the intact rat. The liver, when incubated with [^{14}C]mevalonate *in vitro*, has been shown to be capable of converting mevalonate to sterols at a rate approximately equal to that of the kidneys (21). In fact, as previously demonstrated, at the higher concentrations of circulating mevalonate which might be expected in the nephrectomized animal, the ability of the liver to synthesize sterols can exceed that of the kidney. It seems likely that a combination of these several factors enables the extrarenal tissues, and particularly the liver, to compensate fully for the loss of renal sterologenesis in the anephric rat.

In contrast to the negligible, overall effect of nephrectomy on mevalonate metabolism by the sterol pathway, the loss of the kidneys dramatically decreased the oxidation of mevalonate over the shunt route to 50% of normal. This marked depression in mevalonate shunt metabolism occurred despite the redistribution of blood and labeled mevalonate to other tissues that follows nephrectomy. If these factors are, in part, taken into account by relating shunt activity to the total metabolites produced from circulating mevalonate, nephrectomy produces an average decrease of 63% in the activity of the mevalonate shunt pathway (Table II). These data, therefore, directly demonstrate that the kidneys of the intact rat must be responsible for at least two-thirds of the mevalonate metabolized by the shunt pathway.[2]

In our previous *in vitro* studies (24), the kidneys proved to be the only tissue capable of oxidizing significant amounts of mevalonate by the shunt pathway. The dominant role of the kidney in the shunt pathway of mevalonate metabolism was confirmed in the present study. Nonetheless, despite their lower individual capacities to oxidize mevalonate to CO_2, the various nonrenal tissues, in the aggregate, can account for approximately 37% of the mevalonate metabolized by the shunt pathway.

Edmond and Popják (22) carried out their *in vivo* studies of the shunt pathway in infant rats. It was therefore of importance to determine whether age difference is a factor in the metabolism of mevalonate by the shunt and sterol pathways. The results of our studies demonstrated that there is no significant difference in the metabolism of mevalonate in the young and mature animals, in that the kidney, by *in vitro* assay, represents the predominant site of oxidation of [5-^{14}C]-mevalonate to $^{14}CO_2$, and only an insignificant metabolism of mevalonate by the shunt pathway could be detected in either brain or skin. Moreover, nephrectomy in the infant rat produced an increase in mevalonate metabolism over the sterol pathway and a significant decrease over the shunt pathway, exactly as was observed in the adult animal.

Finally, as emphasized throughout this report, the conclusions regarding the role of the shunt pathway in mevalonate metabolism and the place of the kidneys in this process apply to circulating mevalonate. We have previously demonstrated that the major tissue site of mevalonate synthesis is the liver (20); renal tissue, while very active in its disposal, has only a small capacity to produce this sterol precursor. It follows that the role of the kidney in influencing the overall process of sterol synthesis in the intact animal depends largely upon the amount of mevalonate released into the bloodstream by the liver. We have previously calculated (20), on the basis of a turnover time of approximately 40 min and a blood concentration of 5 μg/100 ml, that in the normal rat 5% of the mevalonate synthesized by the liver reaches the circulation. While changes in the rate of mevalonate metabolism by the nonsterol pathway, which functions primarily in the kidney, may well play a major role in influencing the disposition of circulating mevalonate within the kidney, the present data do not permit the conclusion that the renal shunt or sterol pathways of mevalonate metabolism greatly affect overall sterol metabolism in the normal intact animal. It is possible, of course, in pathologic states such as malignancy, in which the control of mevalonate production by dietary cholesterol is consistently lost (14–16), that these renal pathways of mevalonate disposal may become of greater importance in the overall metabolism of sterols. The possible roles of the renal mevalonate shunt and sterol pathways in such disease states are currently under study.

[2] Since completion of these studies, Edmond *et al.* (26) have presented an abstract reporting that bilateral nephrectomy results in a reduction of exhaled $^{14}CO_2$ from [5-^{14}C]mevalonate to less than half that seen in sham-operated animals. These studies have subsequently been published (27).

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