Mevalonate metabolism by renal tissue in vitro

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Abstract Previous studies from this laboratory have demonstrated that the kidneys rather than the liver play the major role in the in vivo metabolism of circulating mevalonic acid. Kidneys, however, convert mevalonic acid primarily to the precursors of cholesterol, squalene and lanosterol, rather than to cholesterol. This study was designed to define the specific tissue site within the kidney responsible for mevalonic acid metabolism. Tissue slices from rat and dog renal cortex and medulla and glomeruli and tubules were isolated, and the incorporation of ^{14}C-labeled mevalonic acid into the nonsaponifiable lipids squalene, lanosterol, and cholesterol was determined in these tissues. The results demonstrate that the renal cortex is the primary site of mevalonic acid metabolism within the kidney and that the glomerulus is responsible for 95% of the mevalonic acid metabolized by the renal cortex. As was the case for the whole kidney, the major metabolites of mevalonate in the glomeruli are squalene and lanosterol.

Supplementary key words glomeruli · tubules · squalene · lanosterol · cholesterol · renal cortex · renal medulla

Previous studies from this laboratory have presented evidence that the kidneys rather than the liver play the major role in the in vivo metabolism of circulating mevalonic acid (1). These experiments demonstrated that after the intravenous administration of DL-[2-^{14}C]mevalonate, fully 32% of the L-mevalonate was metabolized by the kidney while less than 8% was present in the liver. Moreover, renal metabolism of mevalonate was found to involve primarily the conversion of mevalonate to squalene and lanosterol with relatively little synthesis of cholesterol. It is apparent, therefore, that the kidneys represent a heretofore unsuspected site of mevalonate metabolism and of sterol synthesis in the body.

It was the purpose of the present study to examine in vitro the renal metabolism of mevalonate in greater detail and to define the specific tissue site within the kidney that is responsible for this process. The results reported here demonstrate that the renal cortex is the primary site of mevalonate metabolism within the kidney and that the glomerulus is the tissue responsible for 95% of the mevalonate metabolized by the renal cortex.

MATERIALS AND METHODS

Kidneys and livers were obtained from Holtzman rats and from female mongrel dogs that had been fed a normal laboratory chow ad lib. Rats were stunned by a blow on the head and exsanguinated, and kidneys and livers were rapidly removed. Kidneys from dogs were removed under barbiturate anesthesia. Tissues from both species were handled similarly. Livers and kidneys were placed in cold sodium phosphate buffer, 0.1 M, pH 7.0, immediately after removal from the animal. The renal capsules were promptly stripped and the kidney was bisected. Renal cortex and medulla were separated by sharp dissection. Glomeruli were prepared from the renal cortex by the method of Goodman, Greenspon, and Krakower (2). This procedure involves passing the tissue through 100-mesh screen and allowing the glomeruli repeatedly to sediment in 0.9% saline. Tubules are isolated by centrifugation of the glomeruli-free supernate at 10,000 *g* for 10 min. The resulting pellet contains 95% pure tubules. This method therefore results in a relatively clean separation of glomeruli and tubules with only about 5% cross-contamination. Fig. 1 shows a photomicrograph of the preparations of glomeruli and tubules.

200-mg slices of renal cortex, medulla, and liver were incubated in 5 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, at 37°C for 60 min. Isolated renal glomeruli or tubules were incubated in 2 ml of Krebs-Ringer buffer under identical conditions. 2 μCi of [2-^{14}C]acetate (10 μmoles) or DL-[2-^{14}C]mevalonate (8 μmoles), purchased from New England Nuclear, Boston, Mass., was added to all incubation flasks except those, as indicated, where tracer concentrations of substrate were studied.

Total nonsaponifiable radioactivity was determined in the following manner. The reaction was stopped by addition of 1 ml of 90% potassium hydroxide. The contents of the flask were then transferred to 50-ml screw-top culture tubes. 15 ml of ethanol was added, and saponification was carried out on a steam bath for 2 hr. Nonsaponifiable lipid was then extracted by shaking for 20 min with 25 ml of petroleum ether. The petroleum ether was then washed

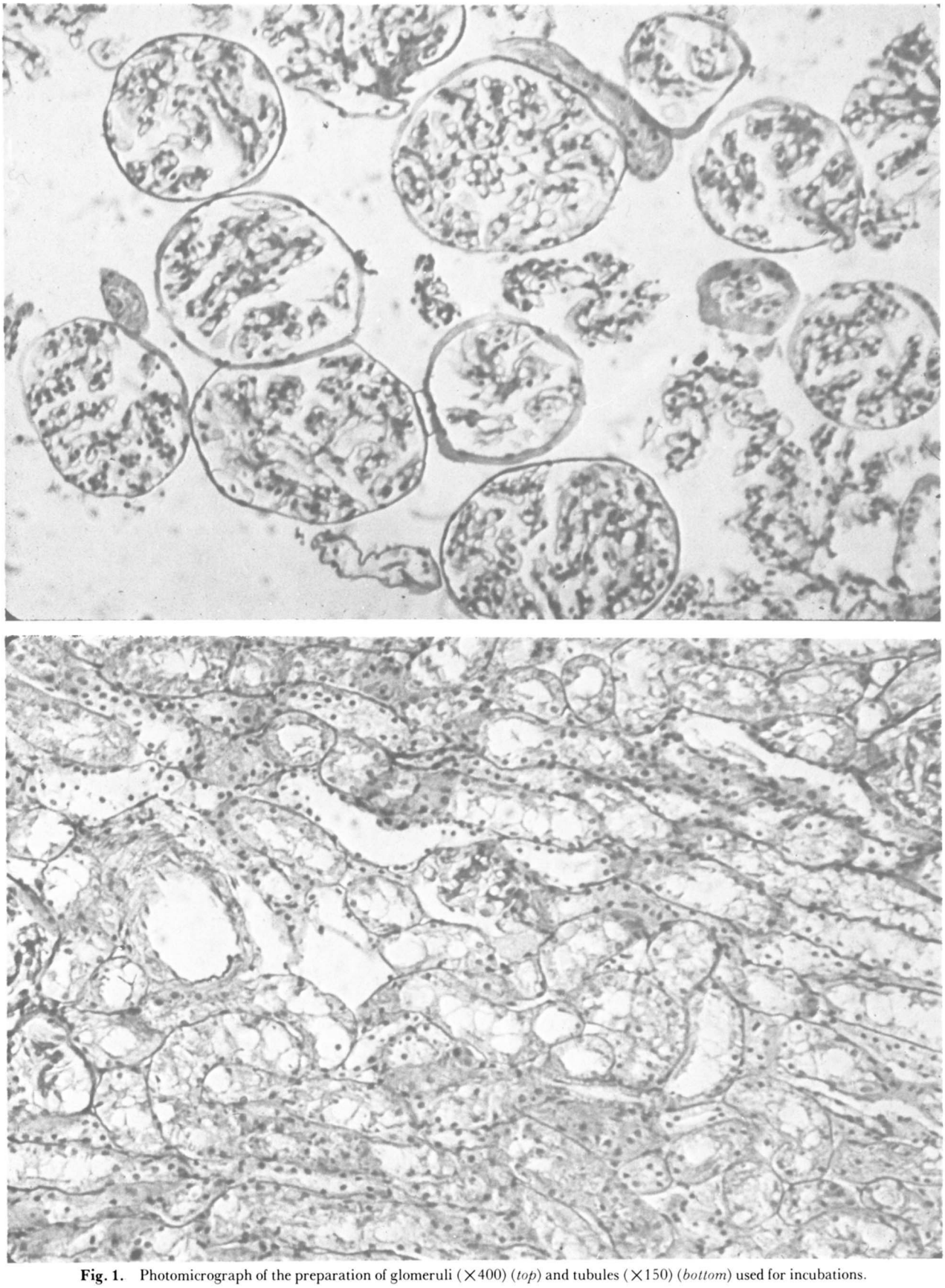

Fig. 1. Photomicrograph of the preparation of glomeruli (×400) (*top*) and tubules (×150) (*bottom*) used for incubations.

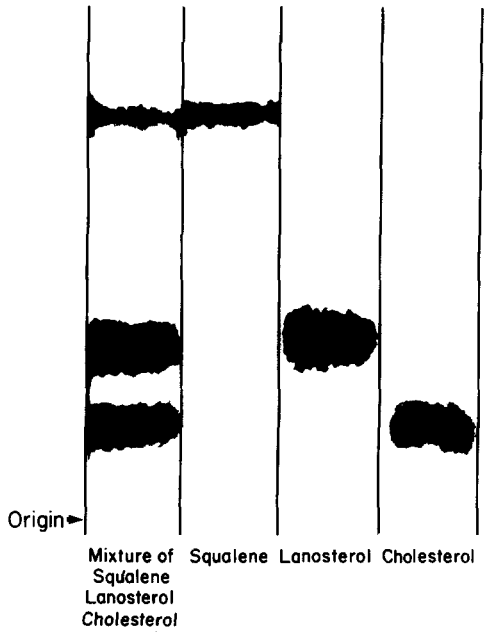

Fig. 2. Thin-layer chromatographic separation of squalene, lanosterol, and cholesterol. The lipids were identified by spraying the plates with a reagent containing 1 ml of *p*-anisaldehyde, 2 ml of H_2SO_4, and 50 ml of acetic acid.

once with 10 ml of 50% aqueous alcohol. The ^{14}C in the nonsaponifiable lipids was determined on a 2-ml aliquot of the washed petroleum ether using *p*-bis-[2-(5-phenyloxazolyl)]-benzene (POPOP) and 2,5-diphenyloxazole (PPO) in ethyl acetate–toluene 6.6:93.4 as the scintillating solution.

Separation of squalene, lanosterol, and cholesterol was accomplished by thin-layer chromatography on silica gel G, 0.25 mm thick on 203 × 203 mm plates. After application of the sample, the plates were first developed in ethyl acetate–benzene 1:5. The solvent front was permitted to ascend about three-quarters of the plate. The plate was then removed from the solvent and completely dried for 60 min under hot air. The plate was then redeveloped in heptane–benzene 97:3, the solvent being permitted to run the entire length of the plate. As can be seen in Fig. 2, this system provides a very clear, sharp separation of cholesterol, lanosterol, and squalene.

RESULTS

Comparison of rat cortex and medulla

As shown in Table 1, the renal cortex of the rat is the primary site of synthesis of nonsaponifiable lipid from mevalonate. Although acetate is a relatively poor precursor of nonsaponifiable lipids, the rat kidney cortex clearly synthesized more nonsaponifiable lipids from acetate than did the medulla.

In experiment 1, the rat cortex converted 319 nmoles of mevalonate/g of tissue to nonsaponifiable lipids as compared with 77 nmoles/g by the medulla. In experiment 2, rat cortex incorporated mevalonate into nonsaponifiable lipids to the extent of 688 nmoles/g, a rate four times that of the medulla (165 nmoles/g). Subfractionation of the nonsaponifiable lipids synthesized by the rat renal cortex and medulla is shown in Table 2. The rat renal cortex converted 40% of the nonsaponifiable lipid synthesized from mevalonate into squalene and 35% into lanosterol, whereas only 19% was incorporated into cholesterol. The medulla also converted [^{14}C]mevalonate primarily into squalene (29%) and lanosterol (40%), with only 24% being incorporated into cholesterol. When the very small amounts of nonsaponifiable lipid synthesized from acetate by the renal cortex and medulla were subfractionated, a somewhat greater conversion to cholesterol was noted.

TABLE 1. Comparison of rat renal cortex and medulla

Expt.[a]	Tissue	Total Nonsaponifiable Lipid	
		From [2-^{14}C]-Acetate	From [2-^{14}C]-Mevalonate
		nmoles/g ± SEM	
1	Cortex	32.0 ± 4.0	319.6 ± 50.0
	Medulla	16.0 ± 2.4	77.0 ± 4.2
2	Cortex	96.9 ± 7.0	688.0 ± 50.0
	Medulla	61.5 ± 4.0	165.0 ± 16.0

[a] Each experiment represents the average of four incubations.

Comparison of dog cortex and medulla

Comparison of mevalonate metabolism by dog cortex and medulla (Table 3) reveals that in the dog, too, the cortex is the primary site of mevalonate metabolism within the kidney. In experiment 1, 147 nmoles of mevalonate/g of tissue were converted to nonsaponifiable lipids by the dog cortex compared with 62 nmoles/g in the dog medulla, and in experiment 2, 91 nmoles of mevalonate/g of tissue were converted into nonsaponifiable lipids by the dog cortex compared with 28 nmoles/g by the dog medulla. Once again acetate was a relatively poor precursor of nonsaponifiable lipid in the dog cortex and medulla.

Table 4 shows the thin-layer analysis of the nonsaponifiable lipid synthesized by the dog cortex and medulla. Of the nonsaponifiable lipids produced from [^{14}C]mevalonate by the dog cortex, 56% was present as squalene, 5% as lanosterol, and only 3% as cholesterol. While the dog medulla synthesized less total nonsaponifiable lipids than the renal cortex, the fractionation of the labeled lipids

TABLE 2. Subfractionation of nonsaponifiable lipids synthesized by rat renal cortex and medulla

Tissue	From [2-^{14}C]Acetate			From [2-^{14}C]Mevalonate		
	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol
	%			%		
Cortex	12	15	25	40	35	19
Medulla	11	11	31	29	40	24

Values are percentages of total ^{14}C-labeled nonsaponifiable lipid.

TABLE 3. Synthesis of nonsaponifiable lipids by dog renal cortex and medulla

Expt.[a]	Tissue	Total Nonsaponifiable Lipid	
		From Acetate	From Mevalonate
		nmoles/g ± SEM	
1	Cortex	27 ± 3	147 ± 12
	Medulla	6 ± 1	62 ± 3
2	Cortex		91 ± 4
	Medulla		28 ± 3

[a] Each experiment represents the average of four incubations.

yielded results similar to those in the cortex, i.e., squalene 59%, lanosterol 13%, and cholesterol 7%. Similar findings were again seen when nonsaponifiable lipids were synthesized with [^{14}C]acetate as substrate.

Mevalonate metabolism by isolated glomeruli and tubules

To localize further the tissue site within the kidney responsible for mevalonate metabolism, renal cortex was fractionated into glomeruli and tubules, and the abilities of these two tissues to convert mevalonate to nonsaponifiable lipids were compared. As shown in Table 5, the glomeruli are clearly the major site of mevalonate metabolism in the kidney; in fact, this tissue was found to convert mevalonate to nonsaponifiable lipids at a rate 20 times that of a comparable amount of tubules.

The results of thin-layer separation of the nonsaponifiable lipids synthesized by the dog glomeruli and tubules are shown in Table 6. 69% of the ^{14}C-labeled nonsaponifiable lipid synthesized by the glomeruli was present as squalene, 4% as lanosterol, and only 5% as cholesterol. A similar distribution of nonsaponifiable lipid was produced by the tubules: 54% of the ^{14}C was in squalene, 12% in lanosterol, and only 6% in cholesterol.

Comparison of cholesterol synthesis in kidney and liver using substrate and tracer concentrations of mevalonate in vitro

It is apparent from the data in Table 7 that rat renal cortical slices converted tracer amounts of mevalonate to nonsaponifiable lipids at a rate consistently greater than liver. In each of the three experiments shown in Table 7, the renal cortex synthesized over three times more nonsaponifiable lipid from mevalonate than did the liver.

TABLE 4. Subfraction of nonsaponifiable lipids synthesized by dog renal cortex and medulla

Tissue	From [2-^{14}C]Acetate			From [2-^{14}C]Mevalonate		
	Squalene	Lanosterol	Cholesterol	Squalene	Lanosterol	Cholesterol
	%			%		
Cortex	27	5	9	56	5	3
Medulla	20	16	20	59	13	7

Values are percentages of ^{14}C-labeled nonsaponifiable lipid.

TABLE 5. Comparison of dog glomeruli and tubles

Expt.[a]		Total Nonsaponifiable Lipid	
		[2-^{14}C]Acetate	[2-^{14}C]-Mevalonate
		nmoles/g ± SEM	
1	Glomeruli	1.2 ± 0.1	27.7 ± 2.6
	Tubules	0.7 ± 0.1	1.2 ± 0.3
2	Glomeruli	3.5 ± 0.2	33.8 ± 1.5
	Tubules	1.2 ± 0.3	0.9 ± 0.1
3	Glomeruli		39.6 ± 2.7
	Tubules		1.9 ± 0.4

[a] Each experiment represents the average of four incubations.

Subfractionation of the nonsaponifiable lipids produced by the renal cortex and liver demonstrated important qualitative differences in the fate of tracer amounts of mevalonate metabolized by these two tissues. As shown in Table 7, cortex, as previously demonstrated in Table 1, converted labeled mevalonate primarily to squalene and lanosterol, with relatively little of the ^{14}C being found in cholesterol. By contrast, in comparable experiments with liver slices, 72 and 66% of the ^{14}C-labeled nonsaponifiable lipid consisted of cholesterol, with squalene and lanosterol together accounting for only 17 and 23% of the total labeled nonsaponifiable lipid. It is apparent, therefore, that renal tissue converts mevalonate to squalene and lanosterol far more readily than does liver, yet in contrast to the liver, the kidney has a limited capacity to carry out the complete synthesis of cholesterol. These results serve to confirm previous findings using whole kidney slices (1).

When rat renal cortex and liver slices are incubated with *substrate* amounts of mevalonate, the resulting synthesis of nonsaponifiable lipid is highly variable (Table 7), with renal cortex synthesizing greater amounts of labeled nonsaponifiable lipids than liver in experiments 2 and 3, and liver exceeding the kidney cortex in experiment 1. When the nonsaponifiable lipids synthesized by the renal cortex and the liver from such substrate amounts of mevalonate were subfractionated, the relative amounts of products produced by these two tissues were quite similar. As expected, the renal cortex converted mevalonate to cholesterol poorly; 42% of the labeled nonsaponifiable lipid was present as squalene, 20% as lanosterol, and only 26% as cholesterol. On the other hand, when incubated with substrate amounts of mevalonate, the liver, too, retained most

TABLE 6. Subfractionation of nonsaponifiable lipids synthesized by dog glomeruli and tubules

Tissue	From Mevalonate		
	Squalene	Lanosterol	Cholesterol
		%	
Glomeruli	69	4	5
Tubules	54	12	6

Each value is the average of quadruplicate analysis. Values are percentages of total ^{14}C-labeled nonsaponifiable lipid.

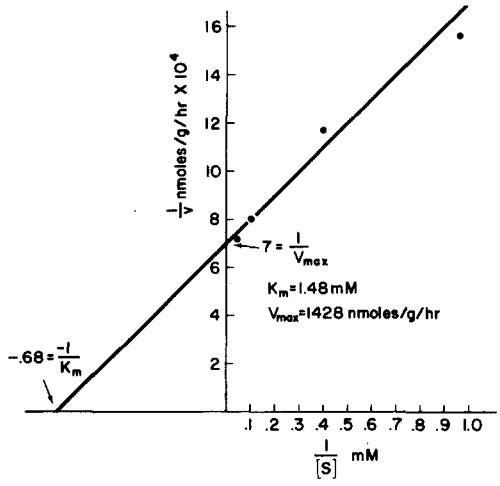

Fig. 3. Squalene synthesis from mevalonate in liver (Lineweaver-Burk plot). 200 mg of liver slices was incubated with increasing concentrations of mevalonate for 60 min at 37°C.

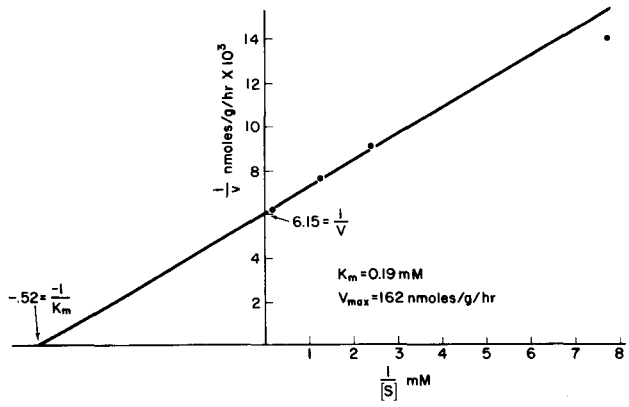

Fig. 4. Squalene synthesis from mevalonate in renal cortex (Lineweaver-Burk plot). 200 mg of renal cortical slices was incubated with increasing concentrations of mevalonate for 60 min at 37°C.

(46 and 51%) of the recovered ^{14}C in squalene and lanosterol, with only 42 and 28% being found in cholesterol.

Figs. 3 and 4 show the Lineweaver-Burk plots from experiments demonstrating the effects of mevalonate concentration on the synthesis of squalene in liver (Fig. 3) and renal cortex (Fig. 4). From these data it is possible to calculate the apparent K_m and V_{max} values of these reactions. For liver, the K_m is 1.48 mM and the V_{max} is 1428 nmoles/g/hr; for the renal cortex, the K_m is 0.19 mM and the V_{max} is 162 nmoles/g/hr.

DISCUSSION

Although previous in vivo and in vitro studies from this laboratory have clearly shown that the kidney plays a major role in the metabolism of circulating mevalonate (1), earlier experiments did not attempt to define the exact tissue site within the kidney responsible for mevalonate metabolism. The present study demonstrates that the renal cortex of both the rat and the dog is capable of converting mevalonate to nonsaponifiable lipids at rates at least four times those of the medulla. Furthermore, when the cortex is fractionated into tubules and glomeruli, over 95% of the mevalonate metabolized by the cortex can be accounted for by the glomerular fraction. These data therefore clearly demonstrate that the renal cortex represents the major site of mevalonate metabolism in the kidney and that the glomerulus represents the major tissue within the kidney responsible for the role that the kidney has been shown to play in sterol metabolism.

As has been previously demonstrated in the intact rat as well as in whole kidney slices (1), metabolism of mevalonate in the kidney cortex and in isolated glomeruli involves the very active conversion of this precursor to squalene and lanosterol with relatively little synthesis of cholesterol. By contrast, liver, incubated with tracer amounts of mevalonate, readily carries out the overall conversion of mevalonate to cholesterol. It is apparent, therefore, that the rate-limiting reaction in the metabolism of mevalonate by renal tissue lies in one or several of the steps between lanosterol and cholesterol. Moreover, at higher levels of mevalonate, a similar rate-limiting reaction can be demonstrated to exist in liver as well; when mevalonate metabolism is studied at substrate concentrations, both liver

TABLE 7. Comparison of sterol synthesis in rat liver and kidney using tracer and substrate amounts of mevalonate

		Tracer (3.9×10^{-5} M)				Substrate (1.6×10^{-3} M)			
Expt.	Tissue	Total Non-saponifiable Lipid	Squalene	Lanosterol	Cholesterol	Total Non-saponifiable Lipid	Squalene	Lanosterol	Cholesterol
		mmoles/g ± *SEM*	%	%	%	*mmoles/g* ± *SEM*	%	%	%
1	Renal cortex	70 ± 4	41[a]	20	26	75 ± 14	42[a]	20	26
	Liver	19 ± 2	12	5	72	250 ± 40	39	7	42
2	Renal cortex	15 ± 2	66	22	5	96 ± 14	53	22	11
	Liver	4 ± 1	12	11	66	64 ± 16	29	22	28
3	Renal cortex	84 ± 6				688 ± 50			
	Liver	20 ± 9				126 ± 7			

[a] Values are percentages of total ^{14}C-labeled nonsaponifiable lipids.

and kidney incorporate the label primarily into squalene and lanosterol rather than into cholesterol.

One minor inconsistency in the data in this study should be noted: Although the renal cortex was shown to metabolize mevalonate at a rate at least three times that of the medulla, small but significant amounts of squalene and lanosterol were in fact produced in slices of the medulla. Since glomeruli are confined to the renal cortex, presumably the tubules of the medulla were responsible for the mevalonate metabolism in medullary slices; yet, when isolated cortical glomeruli and tubules were analyzed separately, at least 95% of mevalonate metabolism took place in the glomeruli, with only negligible synthesis of squalene or lanosterol being observed in the tubules. There are two apparent explanations for this discrepancy in the results. First, it is possible that the tubules of the medulla can more actively metabolize mevalonate than can that portion of the tubules that is in the cortex, or secondly, it is conceivable that the trauma involved in isolating the tubules from the renal cortex causes a disproportionate loss in their ability to metabolize mevalonate. Although we have not carried out further studies to resolve this problem, it is apparent that, whichever explanation is correct, it will not significantly influence the major conclusions of this study, namely, that the glomeruli of the renal cortex represent the major site of mevalonate metabolism in the kidney.

While others have shown that after administration of substrate amounts of mevalonate in vivo the liver is the major site of mevalonate metabolism (3–5), Hellström et al. (1), using tracer quantities of mevalonate, clearly showed that the kidneys metabolize this substrate at much slower rates than liver. These differences between the findings of Hellström et al. (1) and those of all previous investigators are easily explained by the marked differences in the K_m value for the overall reaction of mevalonate to squalene. Although these studies from which K_m was calculated were carried out in tissue slices and the reactions studied, i.e., mevalonate to squalene, represent many enzyme steps, and although the apparent K_m may only be an estimate of the true K_m, it is apparent from these experiments that the renal cortex has a much higher affinity for mevalonate than does liver, the K_m for the renal cortex being one-seventh that of liver. When much higher concentrations of mevalonate are available for metabolism, as would be the case after administration of substrate quantities of mevalonate, the ninefold higher V_{max} of liver would result in greater rates of mevalonate metabolism than would be found in kidney. The tracer quantities of mevalonate used by Hellström et al. (1, 6) more closely approximate the circulating concentration of mevalonate in the rat, which has been shown to be 0.02–0.04 μg/ml. In vivo, therefore, with minute quantities of circulating mevalonate, the renal cortex would be expected to play the major role in mevalonate metabolism.

The physiological significance of the finding that the kidney, and specifically the glomerulus, plays a major role in the conversion of circulating mevalonate to squalene and lanosterol remains to be determined. We have previously established that the products of mevalonate metabolism in the kidney are not secreted into the urine (1), and presumably, therefore, these cholesterol precursors are used for structural purposes, probably within the glomeruli, or else they may be secreted into the renal vein blood for further metabolism elsewhere within the body. The fate of these products of mevalonate metabolism in the kidney is currently under study.

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