# The role of circulating mevalonate in nephrotic hypercholesterolemia in the rat

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**Abstract** The cause of the hypercholesterolemia that characterizes the nephrotic syndrome has never been adequately explained. The present study examines the possibility that enhanced availability of the cholesterol precursor, mevalonic acid, to the liver in the nephrotic state may result in increased hepatic cholesterogenesis. In normal animals, the kidneys are known to be the major site of the metabolism of circulating mevalonate to both cholesterol and $CO_2$. Previous studies, using perfusion of isolated, intact kidneys, have shown that the excretion and metabolism of mevalonate are both impaired in nephrosis. The present investigation has demonstrated in vivo that puromycin aminonucleoside nephrosis results in a 25% reduction in the oxidation of mevalonate to $CO_2$. In the same nephrotic animals, cholesterogenesis from circulating mevalonate was significantly increased in both liver and carcass. In addition, liver slices from nephrotic animals incorporated increased amounts of [5-$^{14}$C]mevalonate into cholesterol when calculated per whole liver, but not per gram of liver. Oxidation of mevalonic acid by kidney slices was significantly reduced, whether expressed as per gram of tissue or per whole organ. HGM-CoA (3-hydroxy-3-methylglutaryl) reductase activity in liver of nephrotic animals was significantly increased. We conclude that, in the nephrotic state, impaired mevalonate metabolism by the kidney may contribute to enhanced cholesterogenesis by increasing delivery of mevalonate to liver and carcass; in addition, nephrosis appears to provide an undefined stimulus for HMG-CoA reductase activity in the liver, thereby providing an additional enhancement of hepatic cholesterogenesis.—**Golper, T. A., K. R. Feingold, M. H. Fulford, and M. D. Siperstein.** The role of circulating mevalonate in nephrotic hypercholesterolemia in the rat. *J. Lipid Res.* 1986. **27:** 1044–1051.

**Supplementary key words** cholesterol • nephrotic syndrome • HMG-CoA reductase • hypercholesterolemia • hyperlipoproteinemia

Hypercholesterolemia is a major feature of nephrotic syndrome in humans and rats. Both species exhibit elevations of very low, intermediate, and low density lipoprotein fractions (1–4). Among the mechanisms responsible for this hyperlipoproteinemia is the increased hepatic synthesis of cholesterol and apoproteins (5–7). Sterol balance studies in nephrotic rats reveal not only an increase in plasma and liver cholesterol, but also total body accumulation of cholesterol (8).

The major site of feedback inhibition of cholesterol synthesis is the production of mevalonate (MVA), a reaction catalyzed by 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase (9). In addition to its incorporation into the sterol pathway, mevalonate is also metabolized by the nonsterol shunt pathway in which MVA carbon atoms contribute to *n*-fatty acids or are oxidized to carbon dioxide (10–15). The kidney is the major organ responsible for the metabolism of circulating MVA into both sterols and nonsterols (14, 16–20). Since elevated levels of circulating MVA favor hepatic cholesterogenesis (12), it is possible that the renal metabolism of MVA to $CO_2$ by the mevalonate shunt pathway may act to divert MVA from hepatic cholesterol synthesis. In addition, Feingold et al. (21) have demonstrated that small amounts (approximately 10–14%) of an administered dose of R-MVA is excreted into the urine over 6 hr.

Edgren and Hellström (22) have observed increased conversion of [$^{14}$C]mevalonate to hepatic sterols in nephrotic rats. Controlling for glomerular filtration rate and hypoalbuminemia, Golper and Swartz (23) demonstrated that isolated perfused kidneys from nephrotic rats metabolized MVA abnormally such that less was excreted, less was oxidized, and less was removed from the recirculating perfusate. Because the kidney has a major role in the metabolism of mevalonate, the impairment of renal metabolism in nephrosis would provide increased amounts of MVA to reach the liver where it could be utilized for cholesterol synthesis. The present experiments were designed to investigate this possibility further.

We have found that a decrease in the oxidation of mevalonate to $CO_2$ does occur in the nephrotic rat in vivo and that there is a concurrent increase in both hepatic and

Abbreviations: MVA, mevalonic acid; HMG, 3-hydroxy-3-methylglutaryl; GFR, glomerular filtration rate.

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carcass cholesterogenesis from mevalonate. Furthermore, in the livers of nephrotic rats, the activity of the rate-limiting enzyme (HMG-CoA reductase) in cholesterol biogenesis is significantly increased at the peak of the diurnal cycle.

## MATERIALS AND METHODS

### Materials

(R,S)-[5-$^{14}$C]mevalonate (15 mCi/mmol) was purchased from Research Products International Corp. (Elk Grove Village, IL). The [1, 2-$^{3}$H]cholesterol (31 Ci/mmol) used as an internal standard was purchased from New England Nuclear. Glucose-6-phosphate (monosodium salt), NADP (yeast $\beta$-NAD), glucose-6-phosphate dehydrogenase (type XXIII from *Leukonostoc mesenteroides*), 2-mercaptoethanol, and $Na_2$ EDTA were purchased from Sigma Chemical Company. Dithiothreitol was purchased from Calbiochem Behringer Corporation. [3-$^{14}$C]HMG-CoA (40–60 mCi/mM) and (R,S)-[5-$^{3}$H]mevalonolactone (5.7 Ci/mmol) were purchased from New England Nuclear. The thin-layer polygram Sil G plates were purchased from Brinkmann Instruments, Inc. (Westbury, NY). The counting solution used for $^{14}CO_2$ and blood samples contained 300 ml of Beckman Bio-Solv III (Beckman Instruments, Fullerton, CA), 1,000 ml of Packard scintillation grade toluene (Packard Instrument Co., Inc., Downers Grove, IL), 100 ml of glass-distilled water, and 6.0 g of 2,5-diphenyloxazole (PPO) (Amersham/Searle Corp., Arlington Heights, IL). The thin-layer strips were counted in Ultrafluor (National Diagnostics). Puromycin aminonucleoside was purchased from Sigma Chemical Co. (St. Louis, MO). Radioautography was carried out on Kodak RP-14 X-ray film (Eastman Kodak Co., Rochester, NY).

### Animal procedures

Female Sprague-Dawley rats (200 g) were purchased from Simonson Animal Vendors (Gilroy, CA). The animals were maintained on a reverse 12-hr light cycle (except where otherwise noted) and were fed Simonson rat and mouse diet and water ad lib. The nephrotic state was induced by puromycin aminonucleoside, 100 mg/kg, as a once only intraperitoneal injection of a 1% solution in 1 N saline. Control animals were injected intraperitoneally with an equal volume of saline. The animals were placed in metabolic cages on day 8 after the puromycin or saline injection and allowed food and water. On day 9, 24-hr urine volumes were collected for protein and creatinine. Food, but not water, was withheld from all animals starting on day 9, unless otherwise noted.

### In vivo studies

Between 8:00–9:00 am on the 10th day (except where otherwise noted) after the intraperitoneal injection, the rats were anesthetized with diethyl ether and injected via the tail vein with 5 $\mu$Ci (1.05 $\mu$mol) of potassium (R,S)-[5-$^{14}$C]mevalonate in 0.4 ml of a 0.9% saline solution. The animals were placed in 2-liter wide-mouth Erlenmeyer flasks fitted with two-hole stoppers. Air was drawn through the bottles at the rate of 2 liters/min, and $^{14}CO_2$ was collected continuously in gas-washing bottles containing 180 ml of 1 N NaOH. To ensure complete trapping of $CO_2$, a second gas-washing bottle also containing 180 ml of 1 N NaOH was connected in tandem with the primary collecting bottle. At the times noted, 0.2-ml samples of the 1 N NaOH were added to 10 ml of scintillation counting solution and the $^{14}$C was determined on a Beckman LS-330 scintillation counter. After 6 hr the animals were killed and weighed, and the livers, kidneys, and carcasses were saponified separately by refluxing overnight in a solution of 45% KOH–$H_2O$–70% ethyl alcohol 2:1:5.

### In vitro studies

The animals were anesthetized with diethyl ether and the liver and kidneys were rapidly removed. Slices of each tissue (0.5-mm thick) were prepared with a McIlwain tissue slicer, and 200 mg of tissue was placed in the outer well of a 25-ml center-well flask containing 2 ml of Krebs-Ringer $PO_4$ buffer and 1 $\mu$Ci (262 nmol) (R,S)-5-[$^{14}$C]mevalonate. The flasks were gassed with 95% $O_2$/5% $CO_2$ for 15 sec, stoppered with serum caps, and incubated for 1 hr at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. At the end of this incubation period, 0.9 ml of 1 N NaOH was injected through the serum cap into the center well and 1 ml of 1 N $H_2SO_4$ was injected through the serum cap into the outer well. $^{14}CO_2$ was then collected in the inner well by reincubating the samples for 15 min in the Dubnoff shaker. The incubation flasks were then opened and 0.1 ml of the center-well NaOH solution was added to scintillation tubes containing 10 ml of counting solution. The $^{14}$C content was determined with a Beckman LS 330 liquid scintillation counter. All values were corrected for the small amounts of $^{14}$C recovered from flasks incubated simultaneously with boiled tissues. The tissue was saponified by refluxing overnight in a solution of 45% KOH–$H_2O$–70% ethyl alcohol 2:1:5 and then analyzed for $^{14}$C-labeled lipids.

### Lipid analysis

The flasks wer cooled and an internal standard of [$^{3}$H]cholesterol was added before extracting the nonsaponifiable material three times with 25 ml of petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and then applied to thin-layer chromatographic plates. The plates were developed in ethyl acetate–benzene 1:5 for 50 min, and the radioactive bands were located by radioautography (3–7 days). The bands corresponding to standards of cholesterol were cut from each plate and placed in scintillation vials containing 10 ml of counting solution and then counted in a Beckman LS 330 scintillation coun-

ter. The gain and discriminator window settings of the scintillation counter were adjusted so that < 0.2% of the $^{3}H$ counts were recorded in the $^{14}C$ window and ~10% of the $^{14}C$ counts were recorded in the $^{3}H$ window. The amount of [$^{3}H$]cholesterol added as an internal standard was adjusted so that $^{3}H$ counts were approximately five times greater than $^{14}C$ counts. Calculations were corrected for spillover of $^{3}H$ and $^{14}C$ and for background. Because only the R isomer of mevalonate is metabolized, all calculations assume that half of the administered mevalonate is inactive.

### Hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity

*Microsomal preparation.* Liver tissue was homogenized (1 g/3 ml) in 0.3 M sucrose, 10 mM 2-mercaptoethanol, 10 mM sodium EDTA, and 50 mM sodium chloride. The homogenate was centrifuged at 10,000 *g* at 5°C for 15 min. The supernatant was next centrifuged at 100,000 *g* at 5°C for 60 min. The resulting microsomal pellet was washed one time with homogenization buffer followed by centrifugation.

*Enzyme assays.* HMG-CoA reductase activity was assayed by incubating 50–200 μg of microsomal protein in 0.2 ml of a solution containing 0.1 M potassium phosphate, 2.5 mM NADP, 12.5 mM DTT, 1.2 units of glucose-6-phosphate dehydrogenase, and [3-$^{14}C$]HMG-CoA (pH 7.4). The mixture was incubated for 1 hr at 37°C and the reaction was terminated by the addition of 10 N HCl. After the incubation of the acidified reaction mixture for 30 min, the [$^{14}C$]mevalonate was separated from HMG-CoA by using a 5.5-cm Bio-Rad Resin Column (AG 1-X8, Formate form, 200–400 mesh). Protein was assayed by the method of Lowry et al. (24).

### [5-$^{14}C$]Mevalonic acid

In the cholesterol synthetic pathway the C-5 of the MVA molecule is always incorporated into the sterol ring. Through the proposed mevalonate shunt pathway carbon-5 of of MVA can become carbon-1 of acetyl-CoA. This carbon may subsequently be oxidized to $^{14}CO_2$ (11, 14). The conversion of (R,S)-[5-$^{14}C$]mevalonate to $^{14}CO_2$ was therefore utilized as the method of measuring mevalonate shunt activity.

### Miscellaneous

Standard urine and blood chemistries were performed in the San Francisco and Portland VA Medical Centers' clinical laboratories.

The statistical analysis employed the *t*-test. The data are expressed as the means ± the standard errors of the mean (SEM). NS refers to a difference that is not statistically significant ($P > 0.05$).

## RESULTS

### In vivo experiments

*Nephrotic syndrome.* Except for ascites, all features of the nephrotic syndrome were demonstrated in the rats that received puromycin aminonucleoside. **Table 1** summarizes the differences between the control and nephrotic animals. The serum cholesterol concentration in the nephrotic rats was greater than three times that seen in the control rats.

*In vivo mevalonate shunt activity.* **Fig. 1** is the plot of shunt activity (nmol of [5-$^{14}C$]MVA converted to $^{14}CO_2$) versus time, for the 6 hr of this in vivo experiment. At each hour the nephrotic animals' shunt activity was significantly lower than that of the controls ($P < 0.005$ at 1 hr and $P < 0.001$ for each time thereafter, two-tailed *t*-test).

Both curves appear to plateau since the rise from 4 to 6 hr is small. If the $^{14}CO_2$ produced from [5-$^{14}C$]MVA is assumed to follow first order kinetics, over 90% of the $^{14}CO_2$ resulting from [$^{14}C$]MVA oxidation was expired during this 6-hr period. Within the nephrotic and control groups, there was no significant correlation between $^{14}CO_2$ production (shunt activity) and creatinine clearance, proteinuria, or serum concentrations of creatinine, albumin, or cholesterol.

*In vivo organ and carcass sterol synthesis from [$^{14}C$]mevalonate.* By 6 h the kidneys of nephrotic animals had converted 203.7 ± 9.2 nmol of administered MVA to non-saponifiable lipids, compared to 217.6 ± 10.1 nmols in

TABLE 1. In vivo experiment. Differences between control and nephrotic animals

| Parameter (Units) | Controls (n = 7) Mean ± SEM | Nephrotics (n = 9) Mean ± SEM | *P* Value |
|---|---|---|---|
| Serum creatinine (mg/dl) | 0.67 ± 0.02 | 1.08 ± 0.07 | < 0.001 |
| Creatinine clearance (cc/min) | 0.714 ± 0.08 | 0.341 ± 0.02 | < 0.001 |
| Proteinuria (mg/day) | 3 ± 1 | 299 ± 31 | < 0.001 |
| Serum albumin (g/dl) | 3.63 ± 0.07 | 2.37 ± 0.08 | < 0.001 |
| Serum cholesterol (mg/dl) | 65 ± 5 | 219 ± 11 | < 0.001 |
| Carcass weight (g) | 185 ± 5 | 161 ± 3 | < 0.01 |
| Wet kidney weight (g) | 1.50 ± 0.04 | 2.33 ± 0.06 | < 0.001 |
| Wet liver weight (g) | 5.41 ± 14 | 6.82 ± 0.13 | < 0.001 |
| Total body weight (g) | 192 ± 5 | 171 ± 3 | < 0.005 |

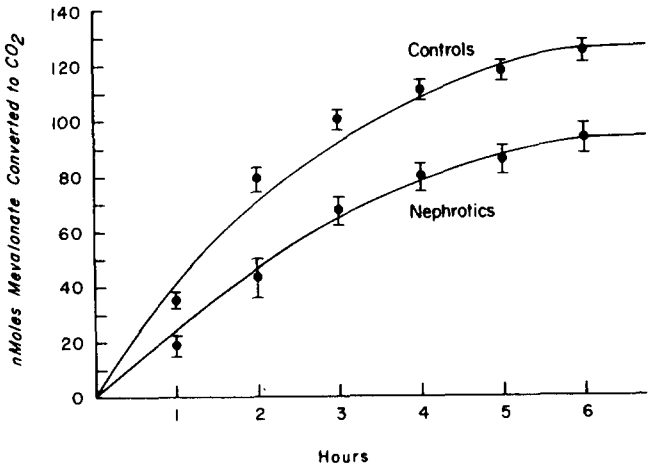


**Fig. 1.** Time course of the oxidation of mevalonate to $^{14}CO_2$. Control and nephrotic rats were injected with 5 μCi (1.05 μmol) of [5-$^{14}$C]MVA. At the times indicated the expired $^{14}CO_2$ trapped in 1 N NaOH was determined.

control animals (NS) (**Table 2**). Mevalonate incorporated into cholesterol in the kidney likewise did not differ in nephrotic and control animals (nephrotics, 170.5 ± 7.8 nmol; controls, 172.4 ± 8.2 nmol). However, in the livers of nephrotic animals, the incorporation of circulating mevalonate into cholesterol and nonsaponifiable lipids was twofold greater than in controls (Table 2). Similarly, in the carcass of nephrotic animals, there was significantly increased incorporation of circulating mevalonate into cholesterol and nonsaponifiable lipids (Table 2). These results demonstrate that while renal cholesterogenesis from mevalonate is not affected by nephrosis, both hepatic and carcass cholesterogenesis are markedly increased.

Of the 500 nmol of the R-[$^{14}$C]MVA injected, we were able to recover 464.1 ± 8.1 nmol (93%) in $^{14}CO_2$ and nonsaponifiable lipids of nephrotic animals and 442.3 ± 13.6 nmols (88%) in control animals (NS). This similarity in the amount of MVA metabolized suggests that the differences seen in the activity of the two pathways of mevalonate metabolism (shunt and sterol) and sites of those activity differences are not due to changes in total MVA metabolized.

## In vitro experiments

*Nephrotic syndrome.* As in the in vivo experiment, the nephrotic animals used in the in vitro studies differed significantly from the control animals in several parameters as summarized in **Table 3.** For both the in vivo and in vitro groups of nephrotic animals, serum albumin concentration inversely correlated with serum cholesterol concentration ($r = -0.686$, $P < 0.001$), while creatinine clearance did not significantly correlate with serum cholesterol concentration ($r = 0.400$).

*Liver slices.* In separate experiments liver slices were incubated with [5-$^{14}$C]MVA and the conversion to $^{14}$C-labeled nonsaponifiable lipids, [$^{14}$C]cholesterol, or $^{14}CO_2$ was determined (**Table 4**).

Per whole liver, the nephrotic animals converted 69.2 ± 5.4 nmol of [5-$^{14}$C]MVA to $^{14}CO_2$, compared to 52.9 ± 4.4 in controls ($P < 0.05$). This difference disappeared when the results were expressed on a per-gram basis. Per whole liver, there was increased cholesterogenesis in the nephrotic rat but this difference, too, did not persist when the results were expressed per gram of tissue. MVA conversion to nonsaponifiable lipids in the liver slices was similar in nephrotic and control animals when measured either per whole organ or per organ weight (Table 4).

*Kidney slices.* [5-$^{14}$C]MVA metabolism was evaluated in kidney slices (**Table 5**). Mevalonate shunt activity (MVA conversion to $CO_2$) was impaired in nephrotic kidneys even when corrected for organ mass. Nephrotic whole kidneys converted 211.3 ± 14.0 nmol of MVA to $CO_2$ while control kidneys converted 254.3 ± 15.3 ($P < 0.05$). Two hundred-mg samples of nephrotic kidney converted 3.81 ± 0.21 nmol of MVA to $CO_2$ while the same mass of control kidneys converted 6.69 ± 0.51 ($P < 0.001$). There was no significant correlation between $^{14}CO_2$ production (nmol/200 mg) and creatinine clearance, $r = 0.317$. Conversion of mevalonate to cholesterol was enhanced in nephrotic kidneys. Cholesterogenesis from [5-$^{14}$C]MVA expressed per kidney was three times greater in nephrotic kidneys than controls ($P < 0.001$), (Table 5). Per renal mass the nephrotic kidneys still displayed a cholesterogenesis rate twice that of controls ($P < 0.005$), (Table 5).

TABLE 2. [5-$^{14}$C]Mevalonate conversion in vivo

| Value | Controls (n = 7) | Nephrotics (n = 9) | *P* Value |
|---|---|---|---|
| nmol of [5-$^{14}$C]MVA converted to | | | |
| Kidney cholesterol | 172.4 ± 8.2 | 170.5 ± 7.8 | NS[a] |
| Kidney nonsaponifiable lipids | 217.6 ± 10.1 | 203.7 ± 9.2 | NS |
| Liver cholesterol | 22.3 ± 1.7 | 45.2 ± 3.0 | 0.001 |
| Liver nonsaponifiable lipids | 25.8 ± 2.3 | 49.0 ± 3.2 | 0.001 |
| Carcass cholesterol | 55.5 ± 4.0 | 98.7 ± 8.4 | 0.001 |
| Carcass nonsaponifiable lipids | 74.6 ± 5.5 | 115.0 ± 9.5 | 0.001 |
| $^{14}CO_2$ (at 6 hr) | 123.6 ± 3.7 | 93.1 ± 4.7 | 0.001 |
| Total [$^{14}$C]MVA metabolized | 442.3 ± 13.6 | 464.1 ± 8.1 | NS |

[a]NS, not significant.

TABLE 3. In vitro experiment. Differences between control and nephrotic animals

| Parameter (Units) | Controls (n = 7) Mean ± SEM | Nephrotics (n = 11) Mean ± SEM | *P* Value |
|---|---|---|---|
| Serum creatinine (mg/dl) | 0.57 ± 0.04 | 1.22 ± 0.06 | < 0.001 |
| Creatinine clearance (cc/min) | 0.761 ± 0.108 | 1.432 ± 0.024 | < 0.001 |
| Proteinuria (mg/day) | 1 ± 0.4 | 292 ± 10 | < 0.001 |
| Serum albumin (g/dl) | 3.52 ± 0.08 | 2.26 ± 0.06 | < 0.001 |
| Serum cholesterol (mg/dl) | 57 ± 3 | 270 ± 7 | < 0.001 |
| Wet kidney weight[a] (g) | 1.53 ± 0.06 | 2.21 ± 0.05 | < 0.001 |
| Wet liver weight[b] (g) | 5.73 ± 0.19 | 7.66 ± 0.14 | < 0.001 |

[a]Since only one kidney was needed, the contralateral kidney was weighed, then dried for 48 hr in the glassware dryer. The wet weight and dry weight correlation coefficient is 0.944.

[b]In experiments where whole livers were not utilized for study, aliquots were weighed wet and dried as above. In control animals, the dry liver weight was 47.2 ± 1.8% of the wet liver weight (n = 6) and in the nephrotic animals, the dry liver weight was 47.3 ± 1.7% of the wet liver weight (n = 6).

Mevalonate conversion to nonsaponifiable lipids in nephrotic kidneys was also greater than controls on a per organ basis (147 ± 8 nmol/nephrotic organ vs. 91 ± 4.8 nmol/control organ ($P < 0.001$), but this difference disappeared when analyzed on a per weight basis, (Table 5).

### 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity

HMG-CoA reductase activity was determined in control and nephrotic rats under a variety of conditions. In fed animals (not fasted the day before or the day of killing) HMG-CoA reductase activity measured at midnight, the peak of the diurnal cycle of reductase activity, was increased in nephrotic animals (nephrotic, n=5, 972 ± 76 vs. controls, n=5, 713 ± 68 pmol of MVA formed/mg of microsomal protein per min, $P < 0.05$). In contrast, HMG-CoA reductase activity measured at 9 AM, which is close to the nadir of reductase activity, was the same in fed control and nephrotic animals (nephrotic, n=6, 413 ± 28 vs. control, n=6, 445 ± 48 pmol of MVA formed/mg of microsomal protein per min, NS). Similarly when both control and nephrotics were fasted for one day, which decreases hepatic HMG-CoA reductase activity, reductase activity measured at midnight was similar in control and nephrotic rats (nephrotics, n=10, 242 ± 22 vs. controls, n=8, 205 ± 22 pmol of MVA formed/mg of microsomal protein per min, NS). Thus, HMG-CoA reductase activity is increased in nephrotic animals when determined at the peak point in the diurnal cycle, but not when measured near the nadir of the cycle or in fasted animals. Six days after the puromycin aminonucleoside injection the animals are just beginning to develop a nephrotic condition. At midnight of the 6th day HMG-CoA reductase activity was not different in nephrotic from controls (nephrotic rats, n=6, 1193 ± 83 vs. control rats, n=6, 1009 ± 83 pmol of MVA/mg of microsomal protein per min, NS).

It should be noted that these results are expressed per mg of microsomal protein. When one takes into account the increased liver size in nephrotic animals (Tables 1 and 3), HMG-CoA reductase activity is increased in both fasted and fed animals at the peak of the diurnal cycle. For example, in fasted animals HMG-CoA reductase activity at the peak of the diurnal cycle in the ten nephrotic animals was 27.91 ± 3.16 nmol of MVA formed/whole organ per min, while in eight control animals it was 13.16 ± 0.92 nmol of MVA formed/whole organ per min ($P < 0.001$). This increase in HMG-CoA reductase activity noted at the peak of the diurnal cycle was not demonstrable at the nadir. For example, even when expressed on a total organ basis, HMG-CoA reductase activity was similar in fed nephrotic and control animals (nephrotic, n=6, 13.60 ± 8.4 vs. control, n=6, 14.02 ± 1.47 nmol of MVA formed/whole organ per min, NS).

TABLE 4. [5-$^{14}$C]Mevalonate conversion by liver slices

| Value | Controls (n = 7) | Nephrotics (n = 11) | *P* Value |
|---|---|---|---|
| [5-$^{14}$C]MVA conversion by liver slices to: | | | |
| $^{14}CO_2$, nmol/organ | 52.9 ± 4.4 | 69.2 ± 5.4 | < 0.05 |
| $^{14}CO_2$, nmol/200 mg | 1.84 ± 0.12 | 1.81 ± 0.13 | NS |
| [$^{14}$C]Cholesterol, nmol/organ | 586 ± 45 | 789.5 ± 72 | < 0.05 |
| [$^{14}$C]Cholesterol, nmol/200 mg | 20.38 ± 1.67 | 20.62 ± 1.83 | NS |
| All $^{14}$C-labeled nonsaponifiable lipids, nmol/organ | 705 ± 59 | 831 ± 74 | NS |
| All $^{14}$C-labeled nonsaponifiable lipids, nmol/200 mg | 24.61 ± 1.88 | 21.70 ± 1.90 | NS |

## DISCUSSION

The etiology of hyperlipidemia in the nephrotic syndrome has recently been reviewed (25). Although the hyperlipidemia is almost certainly multifactorial, the prevailing explanation remains that of Marsh and Sparks (7) and Marsh and Drabkin (26). They demonstrated that in nephrosis the liver responds to hypoalbuminemia by an increase in the synthesis of albumin. They reasoned that the hepatic response to proteinuria is more general and that enhanced lipoprotein synthesis is part of the "general" response, a suggestion that is supported by several investigations (1, 4, 7).

In addition to enhanced lipoprotein synthesis in nephrosis, an increase in cholesterol synthesis has been documented in nephrotic rats and humans (5, 26–28). Although a "general" stimulation of protein synthesis caused by hypoalbuminemia may explain the increased apoprotein synthesis, it does not adequately account for the accelerated hepatic cholesterogenesis of nephrosis. Ohta and Matsuda (29) found no correlation between the degree of hypoalbuminemia and various apoprotein levels. Furthermore, correction of nephrotic hypoalbuminemia may not normalize hypercholesterolemia (3, 30). This observation has led us and others (5, 19, 22, 23, 31) to consider another stimulus to enhanced cholesterogenesis in nephrosis. Inasmuch as the kidney is the major organ responsible for the metabolism of physiologic levels of circulating MVA (16, 18, 20), it seemed reasonable to investigate the renal metabolism of MVA in the nephrotic syndrome. Specifically, we asked two questions. Is renal shunt metabolism impaired in nephrosis and, if so, does this lead to increased liver cholesterogenesis? In isolated perfused kidneys controlled for glomerular filtration rate (GFR) and perfusate albumin concentration, Golper and Swartz (23) demonstrated that nephrotic kidneys removed less MVA from the perfusate, excreted less into the urine, and oxidized less to $CO_2$ than did control kidneys. The current investigation sought to demonstrate that such a relationship between nephrosis and MVA metabolism also occurs in vivo and that the result is an environment favoring enhanced hepatic cholesterogenesis.

The results of our in vivo study confirmed the earlier observations of Edgren and Hellström (22) demonstrating that nephrosis results in an increase in hepatic synthesis of cholesterol from circulating MVA. However, in their studies, the mechanism of this phenomenon was not further explored. In the present study, it was shown that nephrosis results in a 25% reduction in the conversion of circulating MVA to $^{14}CO_2$ (Fig. 1). These observations are not artifactual for three reasons: *1*) approximately 90% of the injected [$^{14}$C]MVA was actually recovered in $^{14}CO_2$ and nonsaponifiable lipids; *2*) renal MVA oxidation was similarly impaired in our in vitro studies; and *3*) MVA metabolism by the sterol pathway was not impaired either in vivo or in vitro in the nephrotic kidneys, suggesting that neither delivery of MVA nor the MVA pool size differed between control and nephrotic animals.

In contrast, the conversion of circulating MVA to nonsaponifiable lipids by liver and carcass was markedly stimulated by the nephrotic state (MVA converted to cholesterol increased 2-fold in the liver and 1.6-fold in the carcass). These observations of decreased shunt oxidation and increased cholesterol synthesis in liver and carcass support our hypothesis that alterations in MVA metabolism by the kidney and, specifically, a decrease in renal oxidation of MVA to $CO_2$ may contribute to the enhanced hepatic (and carcass) cholesterol synthesis of the nephrotic syndrome.

The results of examining MVA shunt metabolism by nephrotic kidney slices confirmed our observations in vivo that metabolism by the shunt pathway is decreased in nephrotic kidneys in the absence of in vivo organ interactions. Although, as shown in Table 2, nephrosis had no effect upon cholesterogenesis in the kidney in the intact animal, the in vitro studies shown in Table 5 demonstrate that kidney slices from nephrotic animals incorporated significantly more mevalonate into cholesterol than did the kidneys of normal animals. These differences between slice and in vivo data may be due to the intact circulation in vivo allowing for the removal of newly synthesized cholesterol.

Regardless of the method of the induction of the nephrotic syndrome, the liver is hypertrophied in nephrotic animals (Tables 1 and 3) (5, 26, 32–34). This observation is significant since, in vitro, the livers of nephrotic animals did not manifest enhanced sterologenesis from mevalonate when calculated as [$^{14}$C]MVA incorporated per g of liver (Table 4). However, per whole liver, cholesterogenesis is clearly enhanced in nephrosis.

TABLE 5. [5-$^{14}$C]Mevalonate conversion by kidney slices

| Value | Controls (n = 7) | Nephrotics (n = 11) | *P* Value |
|---|---|---|---|
| [5-$^{14}$C]MVA conversion by kidney slices to: | | | |
| $^{14}CO_2$, nmol/organ | 254.3 ± 15.3 | 211.3 ± 14.0 | < 0.05 |
| $^{14}CO_2$, nmol/200 mg | 6.69 ± 0.51 | 3.81 ± 0.21 | < 0.001 |
| [$^{14}$C]Cholesterol, nmol/organ | 8.55 ± 1.15 | 29.15 ± 2.5 | < 0.001 |
| [$^{14}$C]Cholesterol, nmol/200 mg | 1.17 ± 0.16 | 2.65 ± 0.22 | < 0.005 |
| All $^{14}$C-labeled nonsaponifiable lipids, nmol/organ | 91.0 ± 4.8 | 147.0 ± 8.0 | < 0.001 |
| All $^{14}$C-labeled nonsaponifiable lipids, nmol/200 mg | 11.96 ± 0.66 | 13.33 ± 0.71 | NS |

In vivo we have demonstrated a markedly enhanced cholesterogenesis from circulating MVA in both liver and carcass. This in vivo effect may be to some extent due to liver hypertrophy but may also be the result of increased availability of MVA to the liver of the nephrotic animals. The diversion of MVA from the kidney to the liver or carcass may be, in part, secondary to the impaired shunt metabolism in the kidney, which we have shown both in vivo and in tissue slices. Several other conditions including nephrectomy, pregnancy, and diabetes mellitus are also characterized by an increase in hepatic sterologenesis associated with an impaired renal metabolism of circulating MVA by the shunt pathway (12, 18, 35, 36). Measurement of circulating MVA levels may help confirm the enhanced delivery of MVA to the liver. A reliable assay was not available at the time these studies were performed.

Unfortunately, no nephrotic model in the rat is without some degree of impairment of renal function. In our experiments the GFR of nephrotic animals was about half that of controls. Serum creatinine concentrations in nephrotics were slightly but significantly greater than in controls (Table 1 and 3). Feingold and associates (21) have extensively studied the effects of uremia on the metabolism of circulating MVA and observed that even in the presence of intact kidneys, uremia induces marked alterations in MVA metabolism. Several lines of reasoning suggest that uremia is not the sole explanation for impaired MVA metabolism in nephrosis. In both the in vivo and in vitro experiments there was no significant correlation between the degree of shunt impairment and serum creatinine levels or GFRs of the nephrotic rats. Furthermore, in the isolated perfused kidney studies of Golper and Swartz (23), nephrotic kidneys were compared to GFR-matched control kidneys, yet MVA oxidation was significantly reduced in the kidneys from nephrotic animals. If impaired renal function, i.e., decreased GFR, were the determinant of impaired MVA shunt metabolism, such a correlation should have been observed under the conditions of these experiments.

An increase in HMG-CoA reductase activity in the livers of nephrotic rats was also confirmed in the present study. This finding is consistent with the findings of Gherardi and Calandri (5) of an increase in [$^3$H]water incorporation into cholesterol in nephrotic rats. Uremia has been reported to reduce hepatic HMG-CoA reductase activity slightly in rats (37) so the increased activity of HMG-CoA reductase that we observed in livers of nephrotic rats cannot be ascribed to uremia. Furthermore, Goldberg and associates (34) noted an increased HMG-CoA reductase activity in the livers of rats rendered nephrotic by the administration of anti-kidney serum. In addition to the contribution of circulating MVA to the increased hepatic cholesterol synthesis in nephrosis, there is a further stimulus for cholesterogenesis acting at the site of the rate-limiting enzyme, HMG-CoA reductase. To our knowledge, there are no data to suggest that hypoalbuminemia increases HMG-CoA reductase activity. Nonetheless, nephrosis clearly stimulates both de novo cholesterol synthesis and its rate-limiting enzyme, HMG-CoA reductase, in the liver.

In conclusion, this study has demonstrated that the nephrotic syndrome in rats results in impaired metabolism of MVA by the shunt pathway and this is associated with increased cholesterogenesis in both the liver and carcass. Puromycin aminonucleoside and nephrotoxic serum-induced nephrosis both cause an increase in HMG-CoA reductase activity in the liver. Because studies in normal animals have shown that circulating mevalonate levels are low (16, 20, 38), alterations in the metabolism of circulating MVA induced by nephrosis may not be a major contributor to the expanded cholesterol pool. We feel that the major cause of the expanded cholesterol pool of nephrosis is the increase in hepatic HMG-CoA reductase activity and the concomitant increase in de novo hepatic cholesterogenesis. Further studies should be directed towards identifying the nature of the stimulus acting on hepatic HMG-CoA reductase.

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