The in Vitro Metabolism of Mevalonate by Sterol and Non-sterol Pathways*

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The metabolism of mevalonic acid by both sterol and non-sterol pathways has been evaluated in nine tissues of the rat. An in vitro estimation of the non-sterol, or "shunt," pathway of mevalonate metabolism was made possible by determining the conversion of [2-14C]mevalonate or [5-14C]mevalonate to 14CO2 in tissue slices. In confirmation of our previous results, the kidney was found to play a major role in the metabolism of mevalonate to sterols and sterol precursors. The shunt pathway accounted for a significant percentage of the mevalonate metabolized in kidney, ileum, spleen, lung, and testes, but was of minor importance or undetectable in liver, brain, skin, and adipose tissue.

Kidney, however, proved to be by far the most active tissue site of mevalonate metabolism by the shunt mechanism in that, on an average, renal tissue metabolized (R)-[14C]mevalonate over the non-sterol pathway at a rate that was 21 times that of any other tissue examined. These results indicate that the kidneys are of major importance in the metabolism of mevalonate by each of the known pathways of metabolism of this sterol precursor.

It has been previously demonstrated in this laboratory that, contrary to expectation, the kidneys rather than the liver represent the major site of uptake and metabolism of circulating mevalonic acid (1). This conclusion was based on the observation that, following the intravenous injection of labeled mevalonate, the kidneys converted approximately 4 times as much mevalonate to cholesterol and cholesterol precursors as did the liver (1). Moreover, in vitro studies renal cortex was consistently more active in sterologenesis from mevalonate than was any other tissue of the body (2).

Edmond and Popjak (3) have recently demonstrated that in intact newborn rats, injected mevalonate can serve not only as a precursor for cholesterol but also as a substrate for long chain fatty acids. In addition, [5-14C]mevalonate administered in vitro was shown by Fogelman et al. to be metabolized to 14CO2 (4). On the basis of these findings, these authors have concluded that a non-sterol, or "shunt," pathway of mevalonate metabolism must operate in the body, probably by the conversion of a portion of the mevalonate molecule to acetocacetate. With the in vitro method employed, this alternate pathway of mevalonate metabolism could be shown to be of significance in only three tissues: brain, spinal cord, and skin. Moreover, the mevalonate shunt pathway could not be demonstrated even in these three tissues when they were studied by in vitro techniques.

The finding of a non-sterol pathway of mevalonate metabo-

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METHODS

Animals and Incubation Procedures—Male Sprague-Dawley rats weighing between 150 and 250 g were used throughout these studies. They were maintained on Purina rat chow and water ad libitum. Animals were anesthetized with ether and decapitated, and the organs to be studied were rapidly removed and placed in chilled Krebs-bicarbonate buffer. Slices of each tissue (0.5 mm thick) were prepared with
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a McIlwain tissue slicer. Unless otherwise noted, 200 mg of slices from each tissue were then placed in 25 ml centrifuge tubes containing 2 ml of Krebs-Ringer bicarbonate buffer and 1 μCi (209 nmol) of (R, S)-[2-14C]mevalonate as the potassium salt, specific activity 18 mCi/mmol (American Radiolabeled Chemical Center or [R,S]-[2-14C]mevalonate with specific activity 15 mCi/mmol (Schwarz/Mann). Unless otherwise noted, unlabelled mevalonate was added to yield a final concentration of 0.1 mM. The tubes were then equilibrated and incubated in an 80°C shaker at 100 oscillations/min for either 1 or 2 hours. All experiments were carried out in duplicate, and the individual figures in each case represent the average of these two values.

While, as noted, (R,S)-[14C]mevalonate was employed in this study, it was well established (4) that only the R-isomer of mevalonate is metabolized. For this reason, all calculations of mevalonate conversion to its metabolites assume that one-half of the injected mevalonate is inactive.

In the studies involving the intrarenal localization of the mevalonate shunt, renal glomeruli and tubules were separated as previously described (2). Briefly, the entire rat kidney was forced through a tissue grinder 6 times, and the resulting suspension was centrifuged at 80 x g for 10 min to remove any remaining glomeruli; the 500 x g supernatant was then centrifuged at 100,000 x g to recover the tubular fractions. This procedure has been shown to yield approximately 90% pure preparations of glomeruli and tubules (2). A 3 ml suspension of a purification period of 15 min in the Dubnoff shaker. The incubation fluids were then added, and the samples were further centrifuged at 1,500 x g for 10 min.

The 14C content was determined using a Beckman LS-230 liquid scintillation counter. All values were corrected for the small amounts of 14CO2 released for every 5 atoms of 14C incorporated into cholesterol from [2-14C]mevalonate.

The correction factor for 14CO2 derived from the sterol pathway of mevalonate metabolism can therefore be obtained by multiplying the 14CO2 incorporated from [2-14C]mevalonate into squalene, individual sterols, and carbon dioxide. If the pathway by which mevalonic acid bypasses sterol production involves incorporation of its 2-14C or 5-14C into a carbon source for the Krebs cycle, measurement of the conversion of [2-14C]mevalonate and [5-14C]mevalonate to 14CO2 should provide a sensitive means of detecting this pathway of mevalonate metabolism. The rationale of this approach and the derivation of the formulas employed are presented in Table I.

The conversion of [2-14C]mevalonate to cholesterol, however, also involves the production of 14CO2 during the demethylation of lanosterol to form cholesterol (Table I, D). An estimate of the mevalonate shunt pathway based on 14CO2 production from [2-14C]mevalonate must therefore involve a correction for any 14CO2 produced during cholesterol synthesis. The amount of 14CO2 released during cholesterol synthesis can, however, readily be estimated by simultaneously injecting unlabeled mevalonate. Based on the incorporation of 14C into squalene, individual sterols, and 14CO2, the quantitative significance of mevalonate metabolism by the sterol and shunt pathways can then be approximated by the equations shown in Table I.

No 14CO2 is lost from [5-14C]mevalonate during the incorporation of this molecule into sterols (4, 7). As a result, when [5-14C] mevalonate is used as a substrate, no correction for 14CO2 derived from sterol demethylation is needed in estimating the activity of the shunt pathway.

The estimates of the activity of the mevalonate shunt pathway based on 14CO2 production obviously represent minimum figures in that several tissues, notably the liver, relatively large amounts of acetyl coenzyme A might be incorporated into long chain fatty acids. As indicated below, no error resulting from incorporation of [2-14C]mevalonate into fatty acids was detected in this study. A second obvious technical error in quantifying the mevalonate shunt pathway, again leading to an underestimation of this pathway of mevalonate metabolism, involves the “entrapment” of 14C into the various intermediate pools of the Krebs cycle. No attempt has been made in the present study to correct for any 14CO2 incorporated into these or other metabolic pools.

RESULTS

Mevalonate Metabolism via Sterol and Shunt Pathways—The quantitative significance of the sterol and shunt pathways of mevalonate metabolism in the various tissues studied for each of four experiments is presented in detail in Table II and summarized in Table III.

Consistent with our previous in vitro studies (1, 2), kidney slices containing both cortex and medulla were found to metabolize mevalonate to sterols and sterol precursors at rates that were comparable to that of liver (Table II, line B; Table III, column 2). By contrast, the sterol pathway of mevalonate metabolism in the other tissues studied was, in general, less than one-tenth as active as that in kidney and liver. In Experiments 3 and 4 brain incorporated mevalonate into sterols at rates comparable to those of kidney and liver; in Experiment 2 this figure was negligible. The reason for the inconsistent sterol synthesis in brain was not pursued further.

The differences in the activity of the shunt pathway in these tissues were even more striking (Table II, line F; Table III, column 1). Of all tissues studied, the kidney proved to be by far the most active site of the mevalonate shunt pathway. In each of the three experiments using [2-14C]mevalonate, the renal conversion of mevalonate to 14CO2 via the shunt pathway (Table II, line F) exceeded that in liver by 8- to over 50-fold, with the
TABLE I

Estimation of pathways of mevalonate metabolism

\[
\begin{align*}
&\text{MEVALONATE} \quad \xrightarrow{\text{SQUALENE}} \quad \text{LANOSTEROL} \quad \xrightarrow{(A)} \quad 27\text{-CARBON STEROLS}^* \\
&\quad \xrightarrow{(B)} \quad \text{CO}_2 \quad \text{(SHUNT PATHWAY)} \quad \longrightarrow \quad \text{TOTAL } [^{14}\text{C}] \text{CO}_2 \quad \longrightarrow \quad \text{CO}_2 \quad \text{(STEROL PATHWAY)} \quad \xrightarrow{(C)}
\end{align*}
\]

- \(A = (R)-[^{14}\text{C}]\text{mevalonate metabolized to 27-carbon }[^{14}\text{C}]\text{sterols (nmol/g tissue/hour)}\)
- \(B = (R)-[^{14}\text{C}]\text{mevalonate metabolized to }[^{14}\text{C}]\text{sterols + }[^{14}\text{C}]\text{squalene (nmol/g tissue/hour)}\)
- \(C = (R)-[^{14}\text{C}]\text{mevalonate metabolized to }[^{14}\text{C}]\text{CO}_2 \text{(nmol/g tissue/hour)}\)
- \(D = 0.2 \times A = (R)-[^{14}\text{C}]\text{mevalonate metabolized to }[^{14}\text{C}]\text{CO}_2 \text{by way of sterol pathway (nmol/g tissue/hour)}\)
- \(E = B + D = (R)-[^{14}\text{C}]\text{mevalonate metabolized by sterol pathway (nmol/g tissue/hour)}\)
- \(F = C - D = (R)-[^{14}\text{C}]\text{mevalonate metabolized to }[^{14}\text{C}]\text{CO}_2 \text{by shunt pathway (nmol/g tissue/hour)}\)
- \(G = \frac{F}{D + F} \times 100 = \text{percent of }[^{14}\text{C}]\text{CO}_2 \text{derived from shunt pathway}\)
- \(H = \frac{F}{D + F + B} \times 100 = \text{percent of recovered } (R)-[^{2-14}\text{C}]\text{mevalonate metabolites derived from shunt pathway}\)

\*Includes cholesterol, desmosterol, and \(\Delta^7\)-cholesterol as well as other unsaturated 27-carbon sterols.

This result was confirmed in Experiment 4 in which \([5-^{14}\text{C}]\text{mevalonate was employed as the substrate. In this experiment kidney metabolized mevalonate by the shunt mechanism at a rate 32 times that of liver.}\)

The relative importance of these two pathways of mevalonate metabolism is indicated for each tissue in Table II, lines G and H, and is summarized in Table III, columns 3 to 6. In liver almost all mevalonate was metabolized by way of the sterol pathway, with an average of only 1% of the metabolized mevalonate using the shunt mechanism. In the kidney, by contrast, a significant fraction (98%) of the mevalonate metabolized utilized the shunt pathway. Of the 72% of mevalonate utilizing the sterol pathway in the kidney, the majority (96%) was recovered as sterols and sterol precursors, and in the case of \([2-^{14}\text{C}]\text{mevalonate a calculated 4% was released as }[^{14}\text{CO}_2 \text{derived by the demethylation of lanosterol. Although, as noted above, the absolute quantitative significance of the shunt pathway in the non-renal tissues studied is minor, in comparison with the sterol pathway the shunt mechanism does play a relatively significant role in the ileum, testes, spleen, skin, and lung (Table III, column 3).}\)

If one compares the roles of each of these pathways of mevalonate metabolism in the production of the \([^{14}\text{C}]\text{CO}_2 \text{derived from } [2-^{14}\text{C}]\text{mevalonate} \text{(Table III, columns 5 and 6)}, \text{the difference between liver and other tissues is striking. In liver, an average of only 13% of the }[^{14}\text{C}]\text{CO}_2 \text{was derived by the shunt pathways, whereas in kidney over 90% of the }[^{14}\text{C}]\text{CO}_2 \text{produced from [2-^{14}\text{C}]mevalonate resulted from this route of mevalonate metabolism. Similarly, in spleen, lung, testes, adipose tissue, and ileum, this figure averaged 50% or more.}\)

Effect of Substrate Concentration on Routes of Mevalonate Metabolism—We have previously shown that the relative ability of various tissues to metabolize mevalonate by the sterol pathway is very dependent upon the concentration of mevalonate to which the tissue is exposed (1, 2). To determine whether the role of the kidney in the mevalonate shunt pathway is similarly influenced by mevalonate concentration, a comparison of the two mechanisms of mevalonate metabolism was carried out at concentrations of \((R)\)-mevalonate that were higher (0.22 and 1.95 mM) than those routinely used in the other phases of this study (0.10 mM). It is apparent from the data in Table IV that regardless of the concentration of mevalonate employed, the activity of the shunt pathway in kidney greatly exceeds that in liver. By contrast, the relative importance of these two tissues in the sterol pathway of mevalonate metabolism changes as the concentration of mevalonate increases. At lower mevalonate levels the kidney converts somewhat more mevalonate to sterols and squalene than does the liver; however, as noted previously (2), the \(V_{\text{max}}\) for the conversion of mevalonate to squalene is higher in the liver than in the kidney. Consequently, at high and unphysiological concentrations of mevalonate the liver converts mevalonate to sterols at rates exceeding those of the kidney. These data therefore confirm a previous finding that the kidney is a major site of mevalonate incorporation into sterols when mevalonate is present in low concentrations. On the other hand, at both
TABLE II
Metabolism of mevalonate in various tissues of the rat

| (R)-[1^4C]Mevalonate metabolized | Experiment 1: [5-^14C]mevalonate | Experiment 2: [2-^14C]mevalonate |
|----------------------------------|----------------------------------|----------------------------------|
|                                  | Kidney                           | Liver                            |
|                                  | Liver                            | Spleen                           | Testes                          | Kidney                           | Liver                            | Spleen                           | Lung | Ileum | Brain | Kidney | Liver | Spleen | Lung | Ileum | Brain |
|                                  | nmol/g tissue/hr                 | nmol/g tissue/hr                 |                                  |                                  |                                  |                                  |      |       |       |        |       |        |      |       |       |
| A. To [1^14C]27-sterol           | 15.6                             | 57.6                             | 0.52                            | 1.10                            | 7.6                             | 27.60                           | 0.36 | 1.16  | 0.32  | 0.38   |       |
| B. To [1^14C]sterol and [1^14C]squalene | 74.2                             | 74.6                             | 1.00                            | 3.50                            | 29.6                            | 30.00                           | 0.50 | 3.30  | 0.40  | 0.62   |       |
| C. To total [^13C]O_3            | 33.9                             | 13.1                             | 0.94                            | 1.00                            | 22.9                            | 5.92                            | 0.28 | 0.68  | 0.14  | 0.07   |       |
| D. To ^13CO_3 by way of sterol pathway | 3.0                             | 11.6                             | 0.10                            | 0.22                            | 1.4                             | 5.52                            | 0.08 | 0.24  | 0.06  | 0.08   |       |
| E. By sterol pathway            | 78.0                             | 88.2                             | 1.70                            | 3.80                            | 33.0                            | 41.00                           | 0.64 | 3.50  | 0.48  | 0.72   |       |
| F. By shunt pathway             | 39.9                             | 1.5                              | 0.84                            | 0.78                            | 21.5                            | 0.40                            | 0.20 | 0.44  | 0.08  | 0.08   |       |
|                                  |                                  |                                  |                                  |                                  |                                  |                                  |      |       |       |        |       |        |      |       |       |
| G. Per cent of ^14CO_3 derived from shunt pathway | 91                               | 11                               | 89                              | 78                              | 94                              | 6.8                             | 71   | 65    | 57    | 0      |       |
| H. Per cent of recovered [^14C] mevalonate metabolites derived from shunt pathway | 28                               | 1.7                              | 33                              | 17                              | 39                              | 1.0                             | 24   | 9.5   | 14    | 0      |       |

* No ^14CO_2 is derived from [5-^14C]mevalonate via the shunt pathway (Experiment 4); with this substrate, therefore, lines B and E are identical and lines C, D, and G are omitted.

TABLE III
Summary of roles of sterol and shunt pathways of mevalonate metabolism in various tissues

| (R)-[^14C]Mevalonate metabolized | Recovered (R)-[^14C]mevalonate metabolites | Recovered ^14CO_2 |
|----------------------------------|------------------------------------------|-------------------|
|                                  | derived from                              | derived from      |
| 1 Shunt pathway                 | 2 Sterol pathway                          | 3 Shunt pathway   | 4 Sterol pathway |
| nmol/g tissue/hr               | %                                         | nmol/g tissue/hr | %                       |
| Kidney                         | 20.93 ± 7.57                              | 54.35 ± 18.42     | 28 ± 8.04               | 72 ± 8.04                   | 92 ± 2.30                      | 8 ± 2.30                        |
| Liver                          | 1.06 ± 0.69                               | 63.00 ± 19.38     | 1.0 ± 0.74              | 99 ± 0.74                   | 13 ± 6.74                      | 87 ± 6.74                       |
| Spleen                         | 0.32 ± 0.35                               | 0.90 ± 0.56       | 21 ± 9.29               | 79 ± 9.29                   | 80 ± 9.00                      | 20 ± 9.00                       |
| Lung                           | 0.23 ± 0.16                               | 31 ± 0.95         | 6 ± 3.77                | 94 ± 3.77                   | 53                             | 47                              |
| Ileum                          | 0.24 ± 0.35                               | 1.05 ± 0.59       | 14 ± 14.00              | 86 ± 14.00                  | 69                             | 31                              |
| Brain                          | 0                                        | 33.64 ± 31.38     | 0                       | 100                         | 0                              | 100                             |
| Adipose                        | 0.04                                      | 0.87              | 3                       | 97                          | 50                             | 50                              |
| Testes                         | 0.78                                      | 3.80              | 17                      | 83                          | 78                             | 22                              |
| Skin                           | 0.03                                      | 0.32              | 9                       | 91                          | 43                             | 57                              |

* Average of Experiments 1, 2, 3, and 4.
* Average of Experiments 1, 2, and 3.
* Where standard deviation is not given, the value represents the average of two experiments. See Table II.
* Where standard deviation is not given, the value represents the average of one experiment. See Table II.
trac and substrate concentrations of mevalonate, the kidney remains the predominant tissue site of mevalonate metabolism by the shunt mechanism.

**Incorporation of [2-14C]Mevalonate into Long Chain Fatty Acids**—Edmond and Popjak (3) have emphasized that, at least *in vivo*, the synthesis of long chain fatty acids from mevalonate can be used as an indication of the activity of the mevalonate shunt mechanism. Attempts were therefore made to determine whether [2-14C]mevalonate could be incorporated into fatty acids by any of the tissues that had been shown by the 14CO2 technique to possess the shunt mechanism. No significant 14C was recovered in any of the individual long chain fatty acids isolated by gas liquid chromatography, nor could we detect labeled farnesoic acid in this preparation. The absence of 14C in the long chain fatty acid fraction confirms the findings of Edmond and Popjak and suggests that quantification of the mevalonate shunt pathway by the determination of 14C incorporation into fatty acids represents an insensitive assay of these reactions.

**Comparison of Renal Cortex, Medulla, Glomeruli, and Tubules as Sites of Mevalonate Metabolism**—In view of the finding that the kidney represents the most active tissue site of mevalonate shunt activity, an attempt was next made to determine whether this metabolic pathway is localized primarily in the cortex, medulla, glomeruli, or tubules of the kidney. Slices of cortex and medulla and relatively pure glomeruli and tubules were isolated as previously described (2). They were then incubated separately with [2-14C]mevalonate, and their ability to metabolize mevalonate by way of the sterol and shunt pathways was compared. The results shown in Table V, line B, demonstrate that the sterol pathway of mevalonate metabolism is localized almost exclusively in the glomeruli. Moreover, the sterol synthetic activity in the cortex exceeded that of the medulla by approximately 3-fold. Similar findings were previously reported from this laboratory (2). Per unit weight of tissue, cortex and glomeruli possess 2 to 3 times the shunt activity found respectively in the renal medulla and glomeruli (Table V, line F). The difference between the glomeruli and tubules is therefore smaller than that noted for the sterol pathway. Both glomeruli and tubules apparently play significant roles in mevalonate metabolism via the shunt pathway.

**DISCUSSION**

It has been previously shown in this laboratory (8, 9) and subsequently in several others (10-12) that the reaction responsible for the synthesis of mevalonate represents the primary biochemical site of cholesterol feedback control in the liver. This finding, coupled with the demonstration that this feedback reaction to dietary cholesterol is consistently and uniquely lost in all tumors (13-18), has led to a re-examination of the metabolic fate of mevalonate by both *in vivo* and *in vitro* techniques. Unexpectedly, these studies demonstrated that circulating mevalonate is primarily metabolized not by the liver, as had been previously assumed, but by the kidneys (1, 2). A similar result has been obtained in both mice and rabbits (19). In these initial studies, it could be shown that the kidneys convert mevalonate primarily to squalene and lanosterol and only to a lesser extent to cholesterol. The dominant role of the kidney in the metabolism of mevalonate to sterols and sterol precursors was subsequently confirmed by Edmond and Popjak (3) and by Edmond (20). These investigators, however, also reported that when injected *in vivo* into newborn rats, mevalonate may be metabolized in brain, spinal cord, and skin by way of a shunt pathway that bypasses sterol synthesis. This pathway of mevalonate metabolism did not appear to play a significant role in renal tissue as determined by either the *in vivo* or *in vitro* methods that were employed. However, Edmond and Popjak were unable to demonstrate mevalonate shunt activity in any tissue as assessed by *in vitro* incorporation of [2-14C]mevalonate into fatty acids; hence there remained a possibility that redistribution of labeled fatty acids among various tissues might limit the validity of their *in vivo* approach to evaluating the tissue localization of the mevalonate shunt pathway. Since 14CO2 production from [2-14C]mevalonate or [5-14C]mevalonate does not depend upon the ability of a tissue to synthesize fatty acids, this technique would appear to offer a more sensitive means of assessing the role of the mevalonate shunt *in vitro*.

As summarized in Fig. 1, the results of this approach to quantification of the pathways of mevalonate metabolism clearly demonstrate that the kidney is overwhelmingly the major tissue site of the mevalonate shunt pathway. In fact, each of the other tissues studied possesses, on an average, less than 5% of the “shunt” activity present in the kidney. The dominant role of the kidney in the metabolism of mevalonate by the shunt pathway is, moreover, observed at all concentrations of mevalonate examined (Table IV). This consistent renal localization of the shunt mechanism of mevalonate disposal (Fig. 1A) contrasts with the tissue distribution of the sterol

**TABLE IV**

| Tissue | (R)-Mevalonate concentration | (R)-[2-14C]Mevalonate metabolized by: |
|--------|-----------------------------|--------------------------------------|
|        |                             | Shunt pathway                        |
|        |                             | Sterol pathway                       |
| Kidney | 0.22                        | 22.0                                 |
| Liver  | 0.22                        | 1.7                                  |
| Kidney | 1.95                        | 49.8                                 |
| Liver  | 1.95                        | 10.0                                 |

**TABLE V**

| (R)-[14C]Mevalonate metabolized | Cortex | Medulla | Glomeruli | Tubules |
|---------------------------------|-------|--------|----------|---------|
| A. To [14C]27-sterol            | 15.2  | 10.0   | 8.0      | 3.00    |
| B. To [14C] sterol and [14C]squalene | 96.4  | 35.0   | 35.6     | 4.40    |
| C. To total [14C] CO2           | 19.3  | 8.7    | 8.3      | 2.70    |
| D. To [14C]CO2 by way of sterol pathway | 3.0   | 2.0    | 1.6      | 0.60    |
| E. By sterol pathway            | 99.4  | 37.0   | 36.2     | 5.00    |
| F. By shunt pathway             | 16.3  | 6.7    | 6.7      | 2.10    |
| G. Per cent of [14C]CO2 derived from shunt pathway | 84    | 77     | 81       | 78      |
| H. Per cent of recovered [2-14C] mevalonate metabolites from shunt pathway | 14    | 15     | 16       | 30      |
pathway of mevalonate metabolism. At low concentrations of mevalonate (Fig. 1B), liver slices manifest approximately the same activity of the sterol pathway as does the kidney, while at high concentrations of mevalonate, by both in vivo (1) and in vitro assay (Table IV) the liver actually becomes the most active site of mevalonate metabolism by this mechanism.

It is noteworthy that on a relative basis, the shunt pathway plays a significant role in mevalonate disposal in a number of the tissues studied. Although, as indicated in Table III, columns 1 and 2, mevalonate is metabolized primarily in kidney, it is possible that in the aggregate the small contributions to mevalonate metabolism by non-renal tissues might become of significance in the intact animal.

The failure to find significant levels of mevalonate shunt metabolism in either brain or skin, even when assayed by the "CO₂ procedure employed here, contrasts with Edmond and Popjak's findings of shunt activity in these two tissues (3) when assayed in vivo. This discrepancy may reflect methodologic differences or may simply be due to the fact that Edmond and Popjak carried out their studies in 12-day-old rats in which, by contrast with the adult, sterol synthesis from acetate is known to become of significance in the intact animal.

In our previous studies it was demonstrated that within the kidney the cortex represents the major site of mevalonate metabolism to squalene and sterols; and, in fact, the glomeruli of the renal cortex were shown to be responsible for over 95% of the mevalonate metabolized by this pathway. The shunt pathway, by contrast, appears to be somewhat more evenly distributed between the glomeruli and tubules of the kidney. Per gram of tissue, the tubules carry out mevalonate metabolism by this pathway while both the tubules and glomeruli metabolize mevalonate via the shunt route.

Finally, the physiologic significance of the renal metabolism of mevalonate by either of the biochemical pathways examined in this report is at present unknown. The level of circulating mevalonate has been shown to be subject to physiologic control (24), and the question of how the malignant (13-18) and non-malignant (25-27) alterations in mevalonate production that were previously demonstrated in this laboratory may influence subsequent renal metabolism of mevalonate by the shunt and sterol pathways remains to be determined.

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