Specifically \(^{14}\text{C}\)-labeled mevalonic acids were administered to rats in diabetic ketosis, and the distribution of \(^{14}\text{C}\) was determined in the hydroxybutyric acid each rat excreted. Also, the distributions of \(^{14}\text{C}\) were determined in hydroxybutyric acid formed by slices of livers and kidneys from rats in diabetic ketosis and incubated with the specifically labeled mevalonic acids. The distributions found are in accord with the conversion of mevalonate to hydroxymethylglutaryl-CoA by the shunt pathway proposed by J. Edmond and G. Popjik ([1974] J. Biol. Chem. 249, 66-71). That is, carbon 5 of mevalonate was metabolized to form the carboxyl of acetyl-CoA and carbons 2 and 3 of mevalonate were converted in large measure to hydroxybutyric acid without acetyl-CoA as an intermediate, i.e. the bond between carbon 2 and 3 was not cleaved, while the bond between 1 and 2, traced with \([1,2-^{14}\text{C}]\)mevalonate, was cleaved. Similar distributions of \(^{14}\text{C}\) were found in hydroxybutyric acid excreted by rats in diabetic ketosis administered specifically \(^{14}\text{C}\)-labeled isovaleric acids, isovaleric acid having in its metabolism intermediates common to those in the shunt pathway.

Estimations of the portions of mevalonate metabolized by the nonsterol- and sterol-forming pathways have been made from yields of \(^{14}\text{CO}_2\) and incorporations of \(^{14}\text{C}\) into sterols from \([2-^{14}\text{C}]\)mevalonate or \([5-^{14}\text{C}]\)mevalonate (6). With the assumption that the shunt pathway is the nonsterol-forming pathway active in kidney and using the yields of \(^{14}\text{CO}_2\) from the \(^{14}\text{C}\)-labeled mevalonates as the measure of that pathway, the kidney has been concluded to metabolize mevalonate by the pathway to a much greater extent than does other tissues (6-9).

The purpose of the present study was to determine whether the metabolism of mevalonate in kidney as well as liver is consistent with its conversion to HMG-CoA and, if so, then to determine to what extent in mevalonate’s conversion to HMG-CoA its carboxyl carbon is retained, since carbon 1 of mevalonate is cleaved in the shunt pathway, but not in the alternate pathways considered by Edmond and Popjik (1).

## Experimental Procedures

**Materials**—Female rats of the Sprague-Dawley strain, weighing 200-300 g, were used. They were fed ad libitum. Diabetes was induced in them by intravenous injection of streptozotocin after they had been fasted for 24 h, and then they were maintained with insulin for 9 to 11 days (10). Those used were the ones that developed marked ketosis along with glucosuria after the insulin was discontinued. At killing, the concentrations of glucose (11) and \(\beta\)-hydroxybutyrate (12) in their blood were determined. Glucose averaged 25.4 mm and hydroxybutyrate 3.4 mm.

R.\(S\)-[4-\(^{14}\text{C}\)]Mevalonolactone (60 \(\mu\)Ci/\(\mu\)mol) was purchased from the Amersham Corp., and sodium R,\(S\)-[5-\(^{14}\text{C}\)]mevalonate (15 \(\mu\)Ci/\(\mu\)mol) was from Research Products International Corp., Mount Prospect, Ill. R.\(S\)-[2-\(^{14}\text{C}\)]Mevalonolactone (17 \(\mu\)Ci/\(\mu\)mol) and R-[3-\(^{14}\text{C}\)]mevalonolactone (52 \(\mu\)Ci/\(\mu\)mol) were purchased from New England Nuclear. In addition to the evidence for their purity provided by their manufacturers, the lactones were subjected to thin layer chromatography on silica gel plates using an absolute ethanol:toluene (1:4 by volume) solvent system. Each gave a single spot containing more than 99% of the \(^{14}\text{C}\) applied to its plate (13, 14). Oxidation of a sample of the sodium \([3-^{14}\text{C}]\)mevalonate by the Kuhn-Roth procedure (10) gave acetic acid with \(^{14}\text{C}\) localized to its carboxyl carbon while oxidation of a sample of the sodium \([2-^{14}\text{C}]\)mevalonate gave acetic acid devoid of \(^{14}\text{C}\). The carboxyl carbon of the acetic acid should be derived from carbon 3 and the methyl carbon from carbon 6 of mevalonate.

The \(^{14}\text{C}\)-labeled lactones were hydrolyzed with NaOH to the sodium salts of their acids (15) just before use. There is ample evidence that the \(R\) isomer, but not the \(S\) isomer, of mevalonate is metabolized (10), and mevalonolactone has been reported to be better utilized by liver than its acid (17), although apparently in vivo mevalonolactone is quickly hydrolyzed (15, 18).

D.\(L\)-[2-\(^{14}\text{C}\)]Valine (16 \(\mu\)Ci/\(\mu\)mol) and D.\(L\)-[4-\(^{14}\text{C}\)]valine (39 \(\mu\)Ci/\(\mu\)mol) were also purchased from Research Products International Corp. They were deaminated (19), and the resulting \([2-^{14}\text{C}]\)isonovaleric and \([4-^{14}\text{C}]\)isovaleric acids were purified by partition chromatography using a Celite (Johns-Manville No. 535) column and mixtures of chloroform and butanol equilibrated with 2 M potassium phosphate.
buffer of pH 7.6 (20). These acids gave single spots with the mobility of isovaleric acid when chromatographed on silica gel plates using a chloroform:butanol (92:5:7.5, by volume) solvent system.

In Vivo Experiments—The labeled compounds were injected intraperitoneally into the rats in diabetic ketosis. Urine collection was begun following the injection in 0.2 ml of 0.154 M NaCl, at the specific activity provided by the manufacturer, of one of the mevalonates (5 μCi), a mixture of [1-14C]mevalonate and [2-14C]mevalonate (5 μCi of each), or one of the isovaleric acids (5 μCi). A repeat injection was made 18 h later, and the collection of urine was terminated 18 h after that. The urines from each rat were acidified and extracted continuously with ether. Each ether extract was neutralized with NaOH and evaporated to dryness.

The resulting crude sodium hydroxybutyrate was also purified by partition chromatography (21, 22). It was acidified with H2SO4 and mixed with a small amount of Celite 535, and this mixture was transferred to the top of a column of Celite prepared by pouring a slurry of the Celite in chloroform. The column was then developed through successive additions of chloroform, chloroform plus 5% and plus 10% (by volume) of 1-butanol equilibrated with 2 M H2SO4. Mevalonic acid and/or its lactone, extracted by ether from the acidified urines, eluted in fractions of the 100% chloroform. Hydroxybutyric acid eluted in fractions of the 90% chloroform:10% butanol.

The hydroxybutyric acid, containing between 10,000 and 75,000 dpm when isovaleric acid was injected, was then degraded (23). It was converted to crotonic acid by distillation from sulfuric acid, and the crotonic acid was purified by sublimation and catalytically reduced to butyric acid. Lactic acid, if present in the acidified urine, would have been extracted by ether and eluted in the fractions containing hydroxybutyrate (24), but as shown by a tracing with 14C-labeled lactate, it would not contaminate the crotonic acid. An aliquot of the butyric acid was commercially labeled with 14C, was added, and the hydroxybutyrate was isolated and degraded as just described for incubation of scrape.

RESULTS

Distributions of 14C in the hydroxybutyric acids excreted by the ketotic diabetic rats injected with the 14C-labeled mevalonic and isovaleric acids are recorded in Table I. When the mevalonate was equally labeled in its carbons 1 and 2, the hydroxybutyric acid excreted had on the average, relative to carbon 2, 0.56 times as much 14C in carbon 1 and 0.31 times as much in carbon 3, with somewhat more than twice as much 14C in carbon 2 as in carbon 4. When the mevalonate was labeled only in carbon 2, the distribution was similar to that when its carbon 1 and carbon 2 were equally labeled, except that there was relatively less incorporation into carbons 1 and 3 of hydroxybutyric acid, although still on the average 0.29 and 0.22 times as much as in its carbon 2. With [3-14C]mevalonate as substrate, 14C was localized to carbons 1 and 3 of hydroxy-
hydroxybutyric acid, containing carbons 2, 3, and 6 of the
into hydroxybutyrate (30). Cleavage of the HMG-CoA formed
3, and 6 of mevalonic acid (1) and their incorporation
planations for the formation of CO₂ and fatty acids from
nonsterol-forming pathways (Fig. 1) considered possible ex-
ybutyric acid formed by the liver preparations.
substrates. The specific activity of [2-'14C]mevalonate was 0.29/1.00 (Table I), therefore, indicates that a significant portion of acetocoeic acid formed from mevalonate was formed without acetyl-CoA as an intermediate. This is in keeping with the formation of the acetocoeic acid from the mevalonate via HMG-CoA without cleavage of the bond between carbons 2 and 3. That is, there is activity in carbon 4 when [2-'14C]mevalonic acid and in carbon 1 when [3-'14C]mevalonic acid were administered in accord with some of the acetocoeic acid formed from HMG-CoA being converted to acetoacetyl-CoA (31), with the acetoacetyl-CoA then being cleaved to acetyl-CoA, and the acetyl-CoA then reconverted to acetocoeic acid.

Via reversal of the HMG-CoA reductase-catalyzed reaction or the conversion of mevalonic acid directly to 3-hydroxybutyronacetyl-CoA (see Fig. 1) carbon 1 of mevalonic acid would be retained in the formation of HMG-CoA. Therefore, [1,2-'14C] mevalonic acid would yield [1,2-'14C] hydroxybutyric acid. Via the shunt pathway, carbon 1 is lost as CO₂, so [2-'14C] hydroxybutyric acid would be formed. Therefore, the formation of [2-'14C] hydroxybutyric acid from [1,2-'14C] mevalonic acid by kidney slices (Table II) is in keeping with the reactions of the shunt pathway and eliminates the other pathways as contributing significantly to mevalonate's metabolism in kidney.

That the distributions of '14C in hydroxybutyric acid excreted by the rats given [1,2-'14C] mevalonic acid were similar to those in hydroxybutyric acid formed by liver slices and the perfused liver (Tables I and II) indicates that the hydroxybutyric acid that was excreted was formed primarily in the liver, the principal organ of ketone body formation in the rat. Hydroxybutyric acid formed from [1,2-'14C] mevalonic acid contained '14C in carbon 1 but so did the hydroxybutyric acid formed from [3-'14C] mevalonic acid, although to a lesser extent. Since the specific activities of carbons 1 and 2 of the [1,2-'14C] mevalonic acid were the same and since the ratio of incorporation into carbon 1 to carbon 2 of hydroxybutyrate from [2-
14C] mevalonate was 0.29/1.00 (Table I), if carbon 1 of mevalonate had been completely retained in the formation of HMG-CoA, as an approximation, a ratio of 1.29 would have been expected from [1,2-'14C] mevalonic acid. That it was only 0.56 means that (1.29 - 0.56)/100 = 73% of carbon 1 was lost in the formation of hydroxybutyric acid from mevalonate. This is a minimum value in consideration of the possible explanations (3, 15) that hydroxybutyric acid when [2-'14C] mevalonic acid was administered.

One such explanation would be randomization in the Krebs cycle, i.e. [2-'14C] acetyl-CoA → [2,3-'14C] succinic acid → [2,3-'14C] phosphoenolpyruvate → [1,2-'14C] acetyl-CoA. In the ketogenic diet, conversion of pyruvate to acetyl-CoA would be expected to be minimal. This is evidenced by the
fact that when [2-\textsuperscript{14}C]palmitate, which must yield [2-\textsuperscript{14}C]acet-
yl-CoA in its metabolism, is administered to ketotic diabetic rats, it is negligible. \textsuperscript{14}C in carbons 1 and 3 of the hydrox-
butyrate the rats excrete (29).

Another and more likely explanation is \textsuperscript{14}CO\textsubscript{2} fixation. There are minor pathways by which CO\textsubscript{2} can be fixed to become

\textsuperscript{14}C in carbon 1 of acetate and these could become prominent, when the conversion of the carbons of mevalonate to hydroxybutyric acid via HMG-CoA is relatively small. Indeed, only a relatively small amount of mevalonate is metabolized via the shunt in liver (6) and in accord with this, the amounts of \textsuperscript{14}C incorporated into hydroxybutyric acid in this study were relatively small. The small yields of \textsuperscript{14}C in hydroxybutyric acid may be a function of the compartment in which HMG-CoA is formed from the mevalonate, as well as the small contribution of the shunt pathway to mevalonate's metabolism. Formation of HMG-CoA occurs both in mitochondria and cytoplasm (32), but formation of 3-methylglutaconyl-CoA has been demonstrated only in mitochondria (33), the location of 3-methylglu-
taconyl-CoA hydratase has not been defined (34), and HMG-
CoA lyase is present primarily in mitochondria (35).

Since \textsuperscript{14}CO\textsubscript{2} is formed from every molecule of [1-\textsuperscript{14}C]meva-
lonate metabolized by the shunt or sterol-forming pathway, the quantity of \textsuperscript{14}CO\textsubscript{2} formed from [2-\textsuperscript{14}C]mevalonate in its metabolism must be less than from [1-\textsuperscript{14}C]mevalonate. Therefore, assuming that \textsuperscript{14}CO\textsubscript{2} fixation is primarily responsible for the incorporation of \textsuperscript{14}C from [2-\textsuperscript{14}C]mevalonate into carbon 1 of hydroxybutyric acid, the quantity of \textsuperscript{14}C fixed must then be greater for the same quantity of [1,2-\textsuperscript{14}C]mevalonate as [2-
\textsuperscript{14}C]mevalonate metabolized. This is the reason the estimate that 72\% of the mevalonate metabolized via HMG-CoA pro-
ceded with cleavage of the bond between carbon 1 and 2 of the mevalonate is considered a minimum.

[2-\textsuperscript{14}C]isovaleric acid should be metabolized to HMG-CoA to yield the same distribution of \textsuperscript{14}C as when [4-\textsuperscript{14}C]mevalonic acid is metabolized via the shunt pathway, i.e. [2-\textsuperscript{14}C]dimethylacryl-CoA would be intermediate in the metabolism of both (1, 5). [2-\textsuperscript{14}C]Acetyl-CoA would result on cleavage of the HMG-CoA formed. Hydroxybutyric acid formed from [2-
\textsuperscript{14}C]isovaleric acid would, therefore, be expected to have label in carbons 2 and 4 in the same ratio as that observed in carbons 1 and 3 when [5-\textsuperscript{14}C]mevalonate was administered and this was the case. [4-\textsuperscript{14}C]Isovaleric acid, in its metabolism, yields the intermediate to be expected in the metabolism of [2,6-\textsuperscript{14}C]mevalonic acid via the shunt pathway. In accord with this expectation, \textsuperscript{14}C was localized in carbons 2 and 4 of hydroxybutyric acid (Table I). The greater activity in carbon 2 than carbon 4 is also presumably attributable to condensa-
tion of labeled acetyl-CoA with unlabeled acetoacetyl-CoA by another pathway as well, since for example, [2-\textsuperscript{14}C]mevalonate gave hydroxybutyric acid with a carbon 1 to carbon 2 ratio of 0.29, but a carbon 3 to carbon 4 ratio of 0.22/0.47 = 0.46. That is, acetyl-CoA formed from carbon 1 and 2 of acetoacetyl-CoA derived from [2-\textsuperscript{14}C]mevalonic acid could not have condensed to give the distribution of \textsuperscript{14}C found in carbons 3 and 4 of hydroxybutyric acid. That the incorpora-
tion of \textsuperscript{14}C into carbon 1 of hydroxybutyric acid was negligible when [2-\textsuperscript{14}C]mevalonate was incubated with kidney slices, but not liver slices, is in keeping with the shunt being more active in kidney than liver (6) and, therefore, \textsuperscript{14}CO\textsubscript{2} fixation, to any extent it occurred, not being apparent.

These studies have been made under the condition of dia-
betic ketosis, since our tracing of the pathway depends upon the isolation of hydroxybutyric acid. While the contributions of the shunt pathway to mevalonate's metabolism appear to vary depending on the conditions (36-38), the reactions comprising the pathway presumably are the same under differing conditions.

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Mevalonate's Nonsterol-forming Pathway

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