Metabolism of Mevalonate in Rats and Man Not Leading to Sterols*

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SUMMARY

C-5 of mevalonate appears as CO₂ in the breath of rats and men almost immediately after administration either by injection or by mouth. Adult rats exhaled up to 6.5% of a dose of RS-[5-¹⁴C]mevalonate (13% of the utilizable L-enantiomer) in the breath in 100 min. The [¹⁴C]O₂ was not derived either from the metabolism of cholesterol biosynthesized from [5-¹⁴C]mevalonate or from the metabolism of the unnatural S-enantiomer of mevalonate. The amount of [¹⁴C]O₂ expired in the breath was the same whether the [5-¹⁴C]mevalonate was given intravenously or in a drink of water to man. One normocholesterolemic man dissipated 12%, a mildly non-familial hypercholesterolemic man dissipated 10%, and a familial hypercholesterolemic man dissipated 7% of a dose of [5-¹⁴C]mevalonate in 24 hours (calculated as a percent of the [R]-enantiomer). The observations support the hypothesis of the existence of a metabolic shunt of intermediates of sterol biosynthesis, derived from mevalonate, not leading to sterols.

It has been a generally held view over the last 15 years that mevalonate had no metabolic fate other than the provision of carbon atoms for polyisoprenoid biosynthesis, cholesterol being quantitatively the most important end product of its metabolism in animals (1). Christophe and Popják (2) first drew attention in 1961 to a pathway for the metabolism of allylic prenyl pyrophosphate intermediates of sterol biosynthesis, derived from mevalonate, not leading to sterols. This hypothesis was established metabolic path (6). Cleavage of 3-hydroxy-3-methylglutaryl-CoA by its lyase (6) generates free acetoacetate from its C-3, C-3’, C-4, and C-5 and acetyl-CoA from its C-1 and C-2. By the hypothesis proposed, it was mandatory that C-5 of mevalonate appear in the breath as carbon dioxide within 1 min after the administration of [5-¹⁴C]mevalonate or from the metabolism of the unnatural S-enantiomer of mevalonate. The observations support the hypothesis of the existence of a metabolic shunt of intermediates of sterol biosynthesis, derived from mevalonate, not leading to sterols.

1 C-1 of acetoacetate originates in this cycle from CO₂ by the carboxylation of 3,3-dimethylacrylyl-CoA to trans-3-methylglutaconyl-CoA (3). The only known reaction by which cholesterol biosynthesized from [5-¹⁴C]mevalonate can generate an oxidizable product and give [¹⁴C]O₂ in the breath is the formation of pregnenolone whereby the cholesterol side-chain is cleaved and C-23 becomes the β carbon atom of isocaproyl-CoA.

We report now that C-5 of mevalonate appears in the breath as CO₂ within 1 min after the administration of [5-¹⁴C]mevalonate to adult rats or man in amounts far greater than could be accounted for by the transformation of cholesterol into pregnenolone and explicable only by the hypothesis proposed (3-5) or some variant of it.

MATERIALS AND METHODS

RS-[5-¹⁴C]Mevalonolactone—This preparation was obtained from Schwarz-Mann; its specific activity was 11.0 Ci per mol. Because preparations of mevalonolactone frequently contain a small amount of its dehydration product, the preparation was purified by liquid liquid partition chromatography (7). This layer chro-

1 This substance is indistinguishable chromatographically from the dehydration product of mevalonolactone obtained by heating mevalonolactone at low pressure with fused KHSO₄; 3-methylpent-2-en-5-lactone (H.-L. Ngan and G. Popják, unpublished observation).
mevalonate used was most probably labeled in position 5 as specified by the supplier. Even if the specimen of [14C]mevalonate had been labeled in position 4, the conclusions drawn from our observations would not be affected.

The unreacted [14C]mevalonate remaining in the incubation mixture, after extraction of the unsaponifiable matter, was obtained as follows. The reaction mixture was acidified with H2SO4 to pH 2. Then it was extracted four times with light petroleum (b.p. 30–90°) in order to remove fatty acids. The aqueous residue then was extracted with CHCl3 in a continuous extractor for 4 days. The chloroform extract was evaporated to dryness, and the residue was dissolved in a small volume of water and then was neutralized (final volume, 10 ml). Measured portions of this chloroform extract were analyzed by thin layer chromatography and by gas-liquid radiochromatography (see above). Both methods showed a single radioactive component coincident with mevalonolactone. The total radioactivity thus recovered was 69.23 μCi, very close to the amount expected (73.25 μCi) if all the R mevalonate had been utilized in the incubation. In order to ascertain whether the specimen still contained some utilizable R-mevalonate, 13.2 μCi were added to a 3-ml incubation of the 10,000 X g supernatant of liver homogenate fortified with ATP, NADP, and glucose 6-phosphate (see above). After 3 hours at 37 °C, the unsaponifiable lipids extracted from the reaction mixture contained only 0.05% of the 14C added to the incubation. In a parallel incubation of an identical amount of RS-[14C]mevalonate with the same enzyme preparation 37.4% of the added 14C (74.6% of the utilizable mevalonate) was found in unsaponifiable lipid. We conclude that probably more than 99% of the [14C]mevalonate recovered from the large incubation had the R configuration.

Continuous Measurement of 14CO2 in Breath—Animals injected with 14C-labeled substrates were placed in a plastic cage through which air was drawn at the rate of 5 liters per min into a calibrated ionization chamber connected to a vibrating reed electrometer, the ionization current being recorded (10) continuous monitoring of the breath of one man except that the plastic cage for the animal was replaced by a plastic helmet over the head of the subject (11) and the flow of air through the helmet and ionization chamber was 14.5 liters per min.

Intermittent Measurement of 14CO2 in Breath and Determination of Specific Activity of CO2—Because the continuous monitoring of the breath for several hours causes discomfort to men, we modified our our needs techniques developed to study the absorption of fat and lactose in man (12, 13). Our subjects were asked to breathe at intervals through a two-way valve which was connected to an anesthesia bag. Usually five breaths tightly filled the bag, the mouth of which then was plugged continuously and its contents as analyzed by passing the gas through a drying tube and bubbling it into 1 or 2 ml of 1 M methyl benzylhydroxide (Hyamine) in methanol contained in counting vials until the color of added phenolphthalein was discharged. Thus 1 or 2 mmol of CO2 were trapped in the Hyamine to which seintilization fluid (10 ml of 7.5% solution of 2,5-bis[5'-tert-butylbenzoxazolyl]-2'-thiophen in toluene was added. The 14C was counted in a Packard Tri-Carb scintillation spectrometer, model 3320, with an efficiency of 79.5 or 64% with 1 or 2 ml of Hyamine, respectively. Duplicate samples differed by less than 4%. The efficiency of counting was determined by the addition of standards to the counting vials after each sample had been first counted. The method is simple, reproducible and far more sensitive than the monitoring of air with the ionization chamber.

Determination of 14C in Blood—HCO3−—Blood (5 or 10 ml) drawn into a heparin-treated syringe was injected into distilled water (5 or 10 ml) in a conical flask closed with a rubber cap. When the red cells became lysed 1 ml of 6 N HCl was injected through the rubber cap into the hemolysate; the CO2 was trapped in Hyamine solution spread on shredded filter paper held in a cup attached to the cap of the flask. The flask was shaken at 37 °C for 1 hour when

We tender our apologies to Schwarz-Mann for questioning the authenticity of their product. We hope that their will appreciate that our conclusions would have been false if the specimen of mevalonolactone had been labeled in position 2. Squalene biosynthesized from [2-14C]mevalonate gives by degradation unlabeled butane-1,4-diol.
RESULTS

$^{14}$CO$_2$ in Breath after Administration of [5-$^{14}$C]Mevalonate to Rats—For these experiments, three 250-g male rats were injected intramuscularly with 21 mCi (1.78 mcmol) of RS-[5-$^{14}$C]-mevalonate and were placed immediately into the plastic cage of the $^{14}$CO$_2$-monitor. Radioactive CO$_2$ appeared in the expired air within 1 min after the injection and rose to a maximum at about 45 min after the injection followed by an exponential decline over several hours. By the integration of the records, we found that the rats expired in 100 min up to 6.5% of the $^{14}$C of the total dose corresponding to 13% of the $^{14}$C contained in the R-enantiomer (Fig. 2).

In order to exclude the possibility that the $^{14}$CO$_2$ was generated by the degradation of sterols synthesized by the animals, we gave by mouth to a 260-g rat 9.07 mCi (0.97 mg) of [4$^{14}$C]-cholesterol biosynthesized from [5-$^{14}$C]mevalonate (cf. "Methods and Materials") and dissolved in 0.1 ml of 20% ethanol in olive oil. The breath of the animal then was monitored for 7 hours. No trace of $^{14}$C could be detected in the expired air during that time. Analysis of the organs for [4$^{14}$C]cholesterol 7 hours after the feeding showed that the cholesterol was well absorbed. Table I gives the distribution of [4$^{14}$C]cholesterol in the organs of this rat and of another injected intramuscularly with 10 mCi of RS-[5-$^{14}$C]mevalonate. The amount of [4$^{14}$C]cholesterol in the liver of the cholesterol-fed animal was in fact 4 times greater than in the liver of the animal injected with mevalonate. We conclude, therefore, that $^{14}$CO$_2$ in the breath of the animals injected with RS-[5-$^{14}$C]mevalonate did not arise from the metabolism of the [4$^{14}$C]cholesterol synthesized endogenously.

The possibility remained that the $^{14}$CO$_2$ arose by the metabolism of the unnatural S-enantiomer of the mevalonate. Hence, we injected intramuscularly 10 mCi of S-[5-$^{14}$C]mevalonate (cf. "Materials and Methods") into a 250-g rat and monitored its breath for 2 hours. Only a trace of radioactivity appeared in the breath, too small to be quantitated, and which could have arisen from the metabolism of the small amount of the R-enantiomer present in the injected specimen. The presence of some R-[5-$^{14}$C]mevalonate in the specimen of S-mevalonate was indicated by the incorporation of label into the sterols in the liver and kidneys of the animal; these organs together contained a total of $2.57 \times 10^3$ dpm of $^{14}$C in sterols as compared to $6.03 \times 10^3$ dpm after the injection of 10.6 mCi of RS-[5-$^{14}$C]-mevalonate.

$^{14}$CO$_2$ in Breath and Blood-HCO$_3$ after Administration of [5-$^{14}$C]-Mevalonate to Man—The present experiments and those of Edmond and Popjak (3), and some further studies to be reported later, support the thesis that a part of the intermediates derived from mevalonate normally are diverted onto a metabolic pathway not leading to sterols. As impairments of such a shunt could lead to substantial changes in cholesterol biosynthesis (cf. "Discussion"), we explored the existence of such a metabolic shunt in man.

Informed consent for study was obtained from four men who volunteered for experiments with the [5-$^{14}$C]mevalonate.

The first subject was a 74-year-old man who was given intravenously 300 mCi (25.4 mcmol) of RS-[5-$^{14}$C]mevalonate and whose breath was monitored continuously with the ionization chamber (cf. "Methods and Materials"). $^{14}$CO$_2$ appeared in the breath almost immediately after the injection and continued to rise for about 100 min. The calibrated ionization chamber used for the experiments with the rats was out of order on this occasion and another uncalibrated instrument had to be used. For this reason, we were unable to calculate the total $^{14}$C expired. The experiment confirmed, nevertheless, the existence in man also of a metabolic path of mevalonate not leading to sterols.

In three further subjects, the intermittent sampling of breath was employed (cf. "Materials and Methods") over 24 hours after administration of RS-[5-$^{14}$C]mevalonate. The study was made in the Clinical Research Center of University of California at Los Angeles Hospital.

Two tests, 6 weeks apart, were made on the next subject, a 42-year-old male heterozygote familial hypercholesterolemic whose serum cholesterol levels varied between 400 and 500 mg/100 ml (serum triglycerides, 150 mg/100 ml) and who had severe atherosclerosis. On the first occasion he was given

![Fig. 2. Integral curves of $^{14}$CO$_2$ exhaled by rats after intramuscular injection of RS-[5-$^{14}$C]mevalonate (R-MVA).](http://www.jbc.org/content/237/10/1773.full.png)

**TABLE I**

| Organ            | Total [14C]cholesterol content of organs |
|------------------|----------------------------------------|
|                  | After feeding | After injecting RS-[5-$^{14}$C]mevalonate |
|                  | dpm × 10$^{-3}$ | dpm × 10$^{-3}$ |
| Liver            | 3300          | 730            |
| Kidney           | 57            | 5300           |
| Lung             | 148           | 43             |
| Blood*           | 84            | 17             |

* Measured in disintegrations per min × 10$^{-3}$ per ml.
The observations presented demonstrate that a pathway for the metabolism of mevalonate, or of intermediates derived from it, not leading to polyisoprenoids and sterols exists not only in newborn animals (3), but also in the adult rat and man. The rapid excretion of C-5 of mevalonate in breath as CO₂ is in full accord with the metabolic shunt (“the trans-methylglycyltaconate shunt”) postulated previously (3-5). Further experiments made in animals with mevalonate and other substrates labeled in various positions with H, 14C, and 18O, to be reported elsewhere, give also full support for the existence of this metabolic pathway. The elimination of C-5 of mevalonate as CO₂ is probably proportional to the amount of mevalonate not used for steroid synthesis, but can give only the lowest limit for the extent of the metabolic shunt. It was shown previously (3) that significant amounts of mevalonate carbon appeared also in n-fatty acids. Since respiratory CO₂ is in equilibrium with urea, significant amounts of radioactivity, derived from C-5 of mevalonate, must have been excreted in the urine with urea also.

Our data demonstrate that a pathway for the metabolism of mevalonate and intermediates derived from it and not leading to sterols exists in the rat and man, a pathway not generally recognized before.

The significance of this shunt of mevalonate metabolism is that a defect in it could explain some of the human hypercholesterolemias. For example, the approximate normal synthesis of 3 mmol of cholesterol per day in the liver of an adult man represents 18 mmol of mevalonate. An individual who metabolizes 12% of the mevalonate synthesized daily by an alternative pathway would produce daily about 20.5 mmol and dissipate 2.5 mmol. If 20.5 mmol of mevalonate were the average daily production of this substance but only 7% of it were metabolized on a “shunt,” only 1.4 mmol would be dissipated and an extra 1.1 mmol would become available for cholesterol synthesis. Such a change would lead to an excess production of 0.18 x 365 mmol = 52.4 g of cholesterol in 1 year, more than twice the amount of cholesterol circulating in the blood of a normocholesterolemic individual.

We do not wish to imply from our data that familial hypercholesterolemia, for example, results from an impairment of the metabolic shunt of mevalonate, as the extent and variations of this metabolic pathway remain to be defined in a statistically significant population of normals and abnormals, including persons with familial hypercholesterolemia and other metabolic abnormalities.

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