Studies on Coenzyme Q

PATTERN OF LABELING IN COENZYME Q₉ AFTER ADMINISTRATION OF ISOTOPIC ACETATE AND AROMATIC AMINO ACIDS TO RATS

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The isolation of coenzyme Q₉ from the tissues of mammals by Morton at Liverpool (1) and by Lester, Crane, and Hatefi at Madison (2), and the demonstration of its role in electron transport (3) have created great interest in its biochemical origin. Despite the close structural relationship of this compound (4, 5) to the fat-soluble vitamins E and K, it appears that, unlike these vitamins, coenzyme Q is synthesized in the mammal from relatively simple precursors. Our interest in the biosynthesis of coenzyme Q stemmed from our failure to demonstrate any appreciable fall in hepatic coenzyme Q levels in young rats fed diets devoid of coenzyme Q and the other fat-soluble vitamins for appreciable periods of time (6). On similar diets, α-tocopherol levels in liver could be demonstrated to fall to low levels. Morton (1) and Moore (7) have made similar observations. These findings suggested that coenzyme Q can be synthesized in the tissues of the rat although the possibility of intestinal synthesis by the microflora with subsequent absorption of the compound as in the case of vitamin K remained open.

During the past several years, we have carried out an extensive experimental program to elucidate the biosynthesis of CoQ in the rat under various conditions, including those minimizing the contribution of the intestinal microflora. At the outset (6) it seemed likely in mammals that the isoprenoid side chain was derived from acetate via the now well established pathway for isoprenoid biosynthesis via mevalonate and that the benzoquinone moiety came from a preformed essential aromatic nutrient such as phenylalanine. In this paper we describe the isolation of CoQ₉ and Q₉₀ from rat liver and report the results of studies of the incorporation of acetate-1-¹⁴C, phenylalanine-¹⁴C, phenylalanine-3-¹⁴C, tyrosine-U-¹⁴C, tyrosine-3-¹⁴C, and acetate-2-¹⁴C into one or both of these coenzymes in rat liver. The distribution of the radioactivity between the aromatic nucleus and the side chain in CoQ labeled with ¹⁴C from these precursors was also determined by a semimicro chemical degradation of the radioactive CoQ developed in this laboratory (8). The procedure depends upon low temperature ozonolysis followed by neutral permanganate oxidation of the resulting aldehyde. Levulinylaldehyde as the bis-2,4-dinitrophenylhydrazone was isolated as a product of the side chain. Preliminary reports of these results have appeared (8-12).

EXPERIMENTAL PROCEDURE

Animals—Young male albino rats of the Sprague-Dawley strain (Charles River Breeding Laboratories, Brookline, Massachusetts) weighing 150 to 250 g were used in these experiments. The stock diet was Purina chow which contained 25% protein (N × 6.25) and 5% fat. Purified diets containing 18% casein and 6% lard were also used as indicated (13). Kilogram lots of rat liver for the isolation of CoQ₉ and Q₉₀ were obtained from stock animals on ordinary chow diets. The nature of the diet fed to the animal did not appear to influence the concentration of CoQ or the distribution of CoQ homologues in the liver. In given experiments designed to demonstrate the biosynthesis of CoQ in the absence of the microflora of the intestinal tract, the whole small and large intestine from the first portion of the duodenum to the rectum was removed surgically under Nembutal anesthesia (50 mg per kg). The operation involved the ligation of the portal vein, splenic and mesenteric arteries, resection of the spleen, section of the duodenum between ligatures below the point of entry of the common bile duct, and finally section of the rectum just above the anus. The whole intestinal tract was then removed by blunt dissection and the abdomen closed. Without intervention such animals were observed to survive from 4 to 12 hours.

Vitamin A deficiency was induced in some of the animals used in these experiments in order to increase the incorporation of precursor radioactivity into hepatic CoQ₉ (14). Weanling rats 50 to 60 g in weight were fed the diet of Mayer and Krehl (15) for 2 to 4 weeks at which time they weighed 140 to 180 g and had approximately doubled their hepatic CoQ content.

Chemicals—All solvents were redistilled with the exception of analytical grade diethyl ether which was peroxide-free. All reagents were analytical grade. All isotopic substrates but the β-labeled aromatic acids were obtained from Nuclear-Chicago Corporation, Desplaines, Illinois. The acetate-1-¹⁴C and acetate-2-¹⁴C had specific activities of 2.0 mc per mm. The L-phenylalanine-U-¹⁴C and L-tyrosine-U-¹⁴C had been isolated from Chlorella which had been grown in a medium enriched with
\(^1^C\) and were isolated after hydrolysis by ion exchange chromatography and crystallized to constant specific activity. Their radiochemical purity was attested to by carrier dilution with known authentic \( \Delta \)-phenylalanine and \( \Delta \)-tyrosine, respectively, by paper chromatography in 1-butanol-water-acetic acid, and by paper electrophoresis. The specific activity of the \( \Delta \)-phenylalanine was 10.6 mc per mm; that of \( \Delta \)-tyrosine was 10.0 mc per mm. \( \Delta \)-Phenylalanine-3-\(^1^C\) and \( \Delta \)-tyrosine-3-\(^1^C\) were obtained from New England Nuclear Corporation, Boston, Massachusetts. These compounds were unequivocally labeled in the \( \beta \)-carbon atom by the following procedure (16)\(^2\). A Grignard condensation of the appropriate phenyl magnesium bromide with \(^1^C\)\(_2\)CO\(_2\) was carried out to obtain the corresponding benzoic acid. The carboxyl of this acid was then reduced with lithium aluminum hydride to the corresponding alcohol which was then brominated and allowed to react with ethylacetanilido-cyanooacetate in the presence of sodium ethoxide. The condensation product was then hydrolyzed to yield the desired \(^1^C\)-\( \beta \)-labeled amino acid and crystallized to constant specific activity. The specific activity of the \( \Delta \)-phenylalanine-3-\(^1^C\) used was 4.4 mc per mm; that of \( \Delta \)-tyrosine-3-\(^1^C\) was 10.6 mc per mm. These substrates were administered to rats without isotopic dilution as specified.

**General Procedure for Isolation of Crude CoQ by Alumina Chromatography**—The animals were killed by decapitation and the livers quickly removed into 10 volumes of Bloor's reagent (alcohol-ether, 3:1), and homogenized with a high speed Sonifer for 2 minutes at room temperature. The extract was then filtered and the precipitated protein washed with an additional 10 volumes of Bloor's reagent. The extract was then evaporated to dryness in a rotary vacuum evaporator at 45°. KOH in methanol, 10%, was then added in the amount of 5 ml per g of initial tissue, and the extract was saponified in the presence of 1.2% pyrogallol for 2 hours at 80°. After dilution with an equal volume of water, the mixture was extracted three times with 2 volumes of petroleum ether. The petroleum ether extract was washed with water until alkali-free and then dried with \( \text{Na}_2\text{SO}_4\) at 4°. The clear extract was then evaporated to dryness under reduced pressure and weighed.

The nonsaponifiable fraction was then purified by alumina chromatography according to the method of Heaton, Lowe, and Morton (17). Neutral alumina (Woelm) Brockman grade III (6% water) was used. The size of the column varied with the size of the sample. For a nonsaponifiable fraction from 1 to 5 g of tissue, a column 12 x 60 mm containing 3 g of alumina (Woelm) Brockman grade III was used. For 10 to 20 g of liver, a column 14 x 90 mm containing 5 g of alumina was used, and for large scale isolations columns 30 x 120 mm containing 30 g of alumina were used. A glass wool pad was pressed evenly into the tube to close its lower end. The alumina was suspended in 100 to 200 ml of distilled petroleum ether (b.p. 40-55°), allowed to enter the column in a thin stream, and then was packed by washing with solvent. The sample was introduced in 5 to 15 ml of petroleum ether and the column developed with various solvents as indicated in Table I.

The fraction eluted with 4% of ethyl ether in petroleum ether contained CoQ homologues, and it was purified by repeated chromatography on alumina until "spectral purity" of the mixture of CoQ homologues in rat liver was obtained. Absorbances

**Table I**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Composition of eluent</th>
<th>Volume of eluent (ml)</th>
<th>Principal components of fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum ether</td>
<td>100</td>
<td>Hydrocarbons</td>
</tr>
<tr>
<td>2</td>
<td>2% diethyl ether</td>
<td>100</td>
<td>Coenzymes Q</td>
</tr>
<tr>
<td>3</td>
<td>4% diethyl ether</td>
<td>100</td>
<td>Isoprenoid alcohols</td>
</tr>
<tr>
<td>4</td>
<td>6% diethyl ether</td>
<td>50</td>
<td>Sterols</td>
</tr>
<tr>
<td>5</td>
<td>20% diethyl ether</td>
<td>150</td>
<td>Residue</td>
</tr>
<tr>
<td>6</td>
<td>50% diethyl ether</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*The diethyl ether was mixed with petroleum ether.*

\( \lambda_{\text{max}} \) for the isoprenoid alcohols was 275 m\( \mu \) of approximately \( E_{275}^{\text{max}} \) = 180 and a change in absorption at 275 m\( \mu \) upon reduction of the quinone with potassium borohydride \( (\lambda_{\text{max}} = 275 \text{ m}\( \mu \)) of \( E_{275}^{\text{max}} \) of 155 were routinely obtained. The measurements of absorbance were carried out in a Beckman model DK-1 recording spectrophotometer. This spectrophotometric method was used as a basis of estimating the concentration of CoQ's during the isolation and purification of these quinones, with \( \Delta \)-phenylalanine-3-\(^1^C\) used for preparative work, multiple
samples were spotted on long sheets of paper and the system changed to a hybrid of the two methods mentioned above, namely, Vaseline petroleum jelly-impregnated paper and propanol-water, 4:1. Markers of synthetic CoQ9 and CoQ10 in amounts of 10 µg each are shown. Spots are stained with 1% permanganate.

**Chromatography on Polyethylene Powder**—In order to separate the larger quantities of CoQ9 and Q10 obtained from a total of 2 kg of rat liver, the crude CoQ fraction obtained from the alumina purification was subjected to further chromatography on polyethylene powder (21). Of polyethylene powder (Hostalen W), 40 g were suspended in 150 ml of acetone-water, 75:25, and poured into a tube to produce a column 2.2 x 23 cm. Of crude CoQ, 102 mg were dissolved in 150 ml of acetone-water, 3:1, and percolated onto the column. The column was washed with 1 liter of acetone-water, 75:25, and then 2-liter lots of 76 and 77% acetone, respectively. CoQ9 was eluted with 3 liters of 81% acetone. The next fraction eluted with 83% acetone contained a 1:1 mixture of CoQ9 and CoQ10, and CoQ10 was eluted in essentially pure form by 1 liter of acetone-water, 85:15. The fractions containing only CoQ9 and CoQ10, respectively, were evaporated to dryness separately, taken up in petroleum ether, and rechromatographed on small alumina columns. The respective homologues were then eluted and twice recrystallized from methanol.

**Preparation of Diacetates of CoQ9 and CoQ10 Hydroquinones**—The diacetates of CoQ9 and CoQ10 were prepared by reductive acetylation with zinc and acetic anhydride according to the method of Lester et al. (22). The scale of the preparation varied from 5 to 50 mg, depending on the amount of material available. The reagents were varied in proportion to the amount of reactant. Both synthetic and natural CoQ9 and CoQ10 isolated from rat liver were used. Usually 25 mg of CoQ was dissolved in 1 ml of acetic anhydride; 25 mg of zinc dust were added plus 0.2 ml of freshly distilled trimethylamine to combine with any free acetic acid in the anhydride. The mixture was warmed on the steam bath for 3 to 5 minutes and diluted with 5.0 ml of cyclohexane, filtered through glass wool, and transferred to a small separatory funnel. The cyclohexane phase was washed twice with 2.5 ml of 0.3 x HCl and three times with water. The solvent was distilled off in a vacuum and the semisolid residue dissolved in 3.0 ml of hot absolute ethanol. Crystals of the respective diacetates of CoQ9 and Q10 hydroquinone formed in a few hours of standing at 4° and were filtered off after 24 hours. The respective derivatives from CoQ9 and CoQ10 were recrystallized to constant melting points. In tracer experiments, the diacetates were recrystallized to constant specific activity. The diacetate of CoQ9 hydroquinone melted at 37° and showed a single spot (RF = 0.55) when chromatographed on a reverse phase paper chromatogram impregnated with Vaseline petroleum jelly and developed with propanol-water, 4:1. The diacetate of CoQ10 hydroquinone melted at 40° and likewise showed a single spot (RF = 0.47) in the same reverse phase paper chromatographic system.

**Measurement of Infrared Spectra of CoQ9 and Q10**—The infrared spectra of synthetic CoQ9 and Q10 and natural CoQ10 from rat liver were compared in CCl4 solutions by Dr. Hans Noll (23) of the Department of Microbiology, School of Medicine, University of Pittsburgh. He employed a Beckman model IR-4 spectrophotometer.

**Isotopic Experiments**—The radioactivity substrates were administered intraperitoneally to nonfasted rats in doses of 50 to 100 µc per rat. Ten to twenty animals were treated by injection in a single experiment and killed by decapitation 1 to 3 hours later. In some experiments only the livers were pooled for the isolation of CoQ9 and CoQ10. In others the remaining carcass (devoid of head and intestinal contents) was also used. The incorporation of radioactivity into total carcass CoQ in 3 hours was shown to be 5 or 7 times greater than that found in hepatic CoQ at 14 hours. The yield of CoQ in milligrams was also increased so that the average specific activity of the total carcass CoQ was not appreciably different from that of the liver alone. This improvement in the recovery of radioactivity in CoQ9 from the rat made chemical degradations of the isolated coenzyme feasible. In animals subjected to enterectomy, the isotopic substrates were administered intravenously and the animals killed after 90 minutes. CoQ9 and CoQ10 were isolated only from the liver in these experiments.

Spectrophotometric estimation of the amount of CoQ present in the initial nonsaponifiable extracts was carried out with potassium borohydride as a reductant and at λmax = 275 µM, an E1%275 of 188 as the constant. CoQ was isolated from liver and carcass and purified separately to constant specific radioactivity and spectral purity on Brockman grade III alumina as described above. At this point the two samples were combined and CoQ (80 to 90% of the total CoQ) was isolated by preparative reverse phase paper chromatography, with paraffin oil-impregnated paper and 1-propanol-water, 4:1. Paper chromatography was repeated (at least once) to obtain constant
specific radioactivity of the CoQ9. Rechromatography on alumina sufficed to remove the paraffin, and the specially pure 14C-CoQ9 was then crystallized to constant specific activity with 4 to 8 times its weight of authentic cold CoQ9. The diacete of CoQ9 hydroquinone was then prepared by reductive acetylation as previously described (22). This derivative was also recrystallized to constant specific radioactivity, which was usually achieved in one recrystallization. The percentage of incorporation of administered label into CoQ9 was calculated from the spectrophotometric estimation of amount of CoQ present in the initial nonsaponifiable extract and the specific activity of the finally purified diacetate of CoQ9 hydroquinone. This calculation overestimates the incorporation slightly because of the presence of the initial extracts of 10 to 20% of CoQ9 of lesser specific activity but this error is within the variability in biosynthetic rates found in duplicate experiments and is deemed sufficiently accurate for comparisons of the precursor substrates.

The radiochemically pure diacetate of CoQ9 hydroquinone obtained after administration of given substrates was then degraded by ozonolysis to yield 3',6'-dialcetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (m.p. 137°) as a ring fragment and the bis-2,4-dinitrophenylhydrazone of levulinaldehyde as a side chain derivative. Acetone from the terminal isopropylidene group was isolated as the 2,4-dinitrophenylhydrazone of levulinaldehyde for estimation of total sterol by the method of Schoenheimer (21). Further analysis of the ratios of certain bands in the spectra of natural and synthetic CoQ9 revealed them to be identical with synthetic CoQ9. It also chromatographed (Rf 0.48) identically with synthetic CoQ9 in the Vaseline petroleum jelly-dichloromethane system (19). The empirical analysis was satisfactory for

\[ \text{C}_{47}\text{H}_{60}\text{O}_{4}\]

\[ \text{Found: C 81.98, H 10.64, CH}_3\text{O 7.26} \]

\[ \text{Calculated: C 81.56, H 10.39, CH}_3\text{O 7.81} \]

**RESULTS**

Isolation of CoQ9 and Q10 from Rat Liver—The nonsaponifiable fraction from 2 kg of rat liver was chromatographed on a large column (110 x 25 mm) of 35 g of alumina Brockman grade III. The fraction eluted with 4% diethyl ether contained the yellow CoQ band which rechromatographed on alumina to spectral purity, i.e., \( \lambda_{\text{max}}^\text{absorb} 275 \text{ m\nu} \) (\( \Delta E^\text{min}_{1\text{cm}} = 155 \)) and was recrystallized twice from methanol. Of mixed CoQ9 and Q10, 102 mg were obtained. Analysis of this mixture by paper chromatography showed that 81% was CoQ9 and 19% was CoQ10. This mixture was subjected to further chromatography on polyethylene powder and 40.2 mg of CoQ9 and 5.7 mg of CoQ10 were obtained. The CoQ9 melted at 44-45° (no depression with authentic CoQ9) and had the following spectral characteristics:

\[ \lambda_{\text{max}}^\text{absorb} 275 \text{ m\nu} (E_{1\text{cm}}^\text{min} = 187); \lambda_{\text{min}}^\text{ethanol} 236 \text{ m\nu} (E_{1\text{cm}}^\text{min} = 30); \lambda_{\text{max}}^\text{ethanol} 275 \text{ m\nu} (\Delta E^\text{min}_{1\text{cm}} = 150) \].

This material showed one spot (\( R_f = 0.38 \)) on a reverse phase paper chromatogram impregnated with Vaseline petroleum jelly and developed in 1-propanol-water, 4:1, identical with synthetic CoQ9. It also chromatographed (\( R_f = 0.48 \)) identically with synthetic CoQ9 in the Vaseline petroleum jelly-dichloromethane system (19). The empirical analysis was satisfactory for

\[ \text{C}_{47}\text{H}_{60}\text{O}_{4}\]

\[ \text{Found: C 81.13, H 10.44, CH}_3\text{O 7.20} \]

\[ \text{Calculated: C 81.98, H 10.64, CH}_3\text{O 7.26} \]

**Infrared Absorption of CoQ Homologues**—Study of the infrared spectra of natural and synthetic CoQ9 revealed them to be identical with each other and with spectra previously reported for CoQ9 (21). Further analysis of the ratios of certain bands associated with the nucleus of CoQ9 and CoQ10 to others associated with side chain functions revealed the type of pattern noted for the vitamin K homologues (23). These rates are presented in Table II. The side chain bands employed for the comparison are those at 2800 cm\(^{-1}\) due to CH\(_2\) scissoring, at 1450 cm\(^{-1}\) due to CH\(_2\) scissoring, and at 1390 cm\(^{-1}\) due to CH\(_3\) bending. The nuclear functions employed were C=O and C=C stretching with CH\(_2\) scissoring, and at 1390 cm\(^{-1}\) due to CH\(_3\) bending. The identity of the ratios for natural CoQ9 isolated from rat liver and synthetic CoQ9 is evident. Further, the homologous nature of CoQ9 is shown by the increase in the ratios for this member of the series.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>2000 cm(^{-1})</th>
<th>2000 cm(^{-1})</th>
<th>1690 cm(^{-1})</th>
<th>1690 cm(^{-1})</th>
<th>1450 cm(^{-1})</th>
<th>1450 cm(^{-1})</th>
<th>1390 cm(^{-1})</th>
<th>1390 cm(^{-1})</th>
<th>1160 cm(^{-1})</th>
<th>1160 cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ9 (rat liver)</td>
<td>1.00</td>
<td>1.26</td>
<td>0.59</td>
<td>0.68</td>
<td>0.37</td>
<td>0.43</td>
<td>0.37</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoQ9 (synthetic)</td>
<td>1.14</td>
<td>1.35</td>
<td>0.59</td>
<td>0.68</td>
<td>0.37</td>
<td>0.43</td>
<td>0.37</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoQ10 (synthetic)</td>
<td>1.33</td>
<td>1.60</td>
<td>0.66</td>
<td>0.77</td>
<td>0.42</td>
<td>0.49</td>
<td>0.42</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Microanalyses were carried out by Dr. Carl Tiedeke, Teaneck, New Jersey, and by the Microanalytical Laboratory, Mellon Institute of Industrial Research, Pittsburgh, Pennsylvania.
6.0
FRACTION NUMBER

FIG. 2. Chromatography of nonsaponifiable fraction from livers of rats dosed with 100 µc of acetate-1-14C. Each fraction represents 10 ml of eluate.

7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
FRACTION NUMBER

FIG. 3. Chromatography of nonsaponifiable fraction from livers of rats dosed with 100 µc of phenylalanine-U-14C. Each fraction represents 10 ml of eluate.

The incorporation of radioactivity from Acetate-1-14C and Phenylalanine-U-14C into CoQ9 and Q10 in Rat Liver—The nonsaponifiable fractions from the pooled livers of normal rats given either acetate-1-14C or phenylalanine-U-14C intraperitoneally in total doses of 100 µc and killed in 90 minutes were chromatographed on Brockman's grade II alumina. The recovery of radioactivity in 10-ml fractions eluted from the first alumina column, according to the schedule of Table I, is shown in Figs. 2 and 3 for animals receiving acetate-1-14C and phenylalanine-U-14C, respectively. Three major peaks of radioactivity are noted in each case. These peaks were associated with the hydrocarbons (Fractions 1 to 10), with CoQ homologues (Fractions 20 to 35), and with sterols (Fractions 36 to 55), respectively.

The incorporation of radioactivity into hydrocarbons and CoQ homologues was similar with the two precursors. The incorporation of radioactivity into the sterol fraction, however, was 3.5 times greater with acetate-1-14C than with phenylalanine-U-14C. The second hump of radioactivity appearing in the sterol fraction has not been associated with a discrete sterol. Most of the cholesterol is eluted in Fractions 35 to 45. Gas phase chromatograms of these two sets of fractions on columns with 2% silicone gum (SE 30) at 225° showed little qualitative change in the distribution of sterols. Cholesterol, small amounts of aymosterol, and traces of a C27 fraction occurred in both the sample containing Fractions 35 to 45 and in the one following composed of Fractions 45 to 55.

The change in specific radioactivity of the rat liver CoQ fraction derived from acetate-1-14C upon subsequent purification on alumina is shown in Table III. More radioactive impurities are associated with CoQ fraction derived from acetate-1-14C than with phenylalanine-U-14C (6). This appears to be due to the presence of labeled isoprenoid alcohols associated with the CoQ fraction when acetate-1-14C is the precursor (24). As indicated previously, the crude coenzyme fraction from rat liver was purified to a constant specific radioactivity as indicated by disintegrations per minute per λ 9th mol 275 mµ (Δk₂₄₋₅₂₄). The number of alumina chromatographies required to reach constant specific activity varied with the 14C precursor used, more being required for acetate-1-14C-labeled CoQ than that labeled with phenylalanine-U-14C.

When crude 14C-CoQ from rat liver of constant specific radioactivity and the spectral characteristics on alumina was subjected to preparative reverse phase paper chromatography with paraffin oil-impregnated paper and propanol-water, 4:1, and the CoQ₉ and CoQ₁₀ spots eluted, CoQ₉ was found to have about the same specific activity as the crude CoQ, and CoQ₁₀ was virtually non-radioactive. One-fourth of the radioactivity present in the crude CoQ sample was found in a leading fraction which showed no ultraviolet spectrum for CoQ. Reductive acetylation of the CoQ homologues obtained in this manner yielded the corresponding CoQ hydroquinone diacetate with a specific radioactivity usually within experimental error of that obtained for the purified CoQ. Occasionally, the derivative would show an appreciable drop in specific activity, indicating the necessity of making it the final proof of radiochemical purity. The purification of a typical sample is shown in Table IV. Appreciable losses in total radioactivity are sustained in obtaining a final radiochemi-
only 3.5% of the specific activity of the more abundant homodose of given isotope administered. The incorporation of radioactivity contained no radioactivity (6). The enrichment of radioactivity purified chromatographically to spectral purity, and found to in two of the experiments in which acetate-1-W was given, of all components measured was roughly proportional to the activity into CoQIo as compared with Co&~ was low, averaging

cardiac precursors. Considering the difference in abundance, less than 1% of the radioactivity appearing in Co&~ appeared in Co&g in rat either acetate-I-14C or phenylalanine-U-l% is presented in Table IV. Of the six experiments reported, three were performed in enterectomized rats. Vitamin A was isolated from the livers of rats dosed with

correlation of its label into liver fatty acids. The ratio of specific radioactivity for CoQ to cholesterol averaged 0.48 for acetate-1-14C and 4.31 for phenylalanine-U-14C. This suggested that a dual pathway exists for the incorporation of label from phenylalanine into CoQg, permitting more label to enter the

carrying CoQg was added in the amount of 18.0 mg to make the derivative. Stated yield is calculated back to undiluted CoQg.

carrier yielded a product whose specific activity changed very slowly with repeated crystallizations (14).

The distribution of radioactivity among CoQg, CoQo, cholesterol, and fatty acids isolated from the livers of rats dosed with either acetate-1-14C or phenylalanine-U-14C is presented in Table IV. Of the six experiments reported, three were performed in enterectomized rats. Vitamin A was isolated from the livers of rats in two of the experiments in which acetate-1-14C was given, purified chromatographically to spectral purity, and found to contain no radioactivity (6). The enrichment of radioactivity of all components measured was roughly proportional to the dose of given isotope administered. The incorporation of radioactivity into CoQg as compared with CoQg was low, averaging only 3.5% of the specific activity of the more abundant homologue. Considering the difference in abundance, less than 1% of the radioactivity appearing in CoQg appeared in CoQg in rat liver. The incorporation of radioactivity from acetate-1-14C and phenylalanine-U-14C into cholesterol and fatty acids reflected the extent to which these precursors generated acetyl-CoA and related small molecules. Acetate-1-14C enriched both fatty acids and cholesterol effectively whereas phenylalanine-U-14C was comparatively ineffective, particularly as regards the incorporation of its label into liver fatty acids. The ratio of specific radioactivity for CoQ to cholesterol averaged 0.48 for acetate-1-14C and 4.31 for phenylalanine-U-14C. This suggested that a dual pathway exists for the incorporation of label from phenylalanine into CoQg, permitting more label to enter the CoQ molecule than would be predicted from the isoprenoid pathway alone.

In order to validate a dual pathway for the biosynthesis of the ring and side chain components of CoQ, additional experiments were undertaken with vitamin A-deficient animals in which both liver and carcass were used as a source of coenzyme, and in which the isolated CoQ hydroquinone diacetate degraded to obtain a fully substituted phenylactic acid derivative as a ring fragment and the dinitrophenylhydrazone of levulinic acid as a derivative of the side chain.

The CoQ concentration of livers from vitamin A-deficient animals was more than twice that of controls, viz. 270 ± 45 µg per g, fresh weight, for the vitamin A-deficient animals, and 108 ± 11 µg per g, fresh weight, for the controls (25). The concentrations of CoQ in the carcasses of normal and deficient animals were comparable and averaged about 20 µg per g of carcass (total body minus liver, head, and gastrointestinal contents). The carcass, however, supplied about 2.5 times as much CoQ9 as the liver in the pooled samples of CO&~ subjected to study and degradation in these experiments. The amounts of CoQ found in the total nonsaponifiable fractions, the yield of crude CoQ from the alumina step, and the yield of CoQg after two preparative paper chromatograms in seven isotopic experiments are presented in Table V. Approximately 50% of the CoQg actually present was isolated after two paper chromatograms. The yield of carcass cholesterol ranged from 0.51 to 0.83 mg per g.

It may be seen from Table V that the radioactive CoQg isolated

<table>
<thead>
<tr>
<th>Substance</th>
<th>Rate</th>
<th>Dose</th>
<th>Radioactivity of isolated products</th>
<th>Ratio of CoQg activity to cholesterol activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetate-1-14C</td>
<td>N</td>
<td>50</td>
<td>1,500</td>
<td>1,450</td>
</tr>
<tr>
<td>2. Acetate-1-14C</td>
<td>N</td>
<td>50</td>
<td>1,800</td>
<td>2,920</td>
</tr>
<tr>
<td>3. Acetate-1-14C</td>
<td>E</td>
<td>500</td>
<td>19,900</td>
<td>22,050</td>
</tr>
<tr>
<td>4. Acetate-1-14C</td>
<td>E</td>
<td>500</td>
<td>19,600</td>
<td>24,000</td>
</tr>
<tr>
<td>5. Phenylalanine-U-14C</td>
<td>N</td>
<td>50</td>
<td>4,900</td>
<td>56</td>
</tr>
<tr>
<td>6. Phenylalanine-U-14C</td>
<td>E</td>
<td>50</td>
<td>4,900</td>
<td>76</td>
</tr>
</tbody>
</table>

* N, normal; E, enterectomized rats.
* Rat's weighed 150 to 250 g and 2 to 10 rats were used in each experiment.
* Standard error of mean.
The extent of incorporation of all precursors tested into total carcass CoQ9 was of the same order of magnitude, the variation being from 0.005 % with L-phenylalanine-U-14C and lowest with phenylalanine-3-14C, phenylalanine-U-14C, and tyrosine-1-14C.

Table VI presents the results of degradation of the radioactive CoQ9 hydroquinone diacetate isolated in each experiment. The radioactivity reported for the CoQ9 hydroquinone diacetate is the radioactivity of the sample which was degraded. It may be seen that the substrates used fall into two categories, those which predominantly label the ring fragment and those which predominantly label the side chain. In agreement with previous experiments and hypotheses, acetate-1-14C and acetate-2-14C, intermediate with tyrosine-3-14C, and lowest with phenylalanine-3-14C, phenylalanine-U-14C, and tyrosine-1-14C.

Table VI presents the results of degradation of the radioactive CoQ9 hydroquinone diacetate and its degradation products after administration of given radioactive precursors.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Total dose of substrate</th>
<th>CoQ9 hydroquinone diacetate</th>
<th>Degradation products</th>
<th>Distribution of radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phylacetic acidc</td>
<td>Levulinaldehydec</td>
</tr>
<tr>
<td>1. Acetate-1-14C</td>
<td>1.0</td>
<td>13.87 Seeking X = 10P 0.76</td>
<td>1.76</td>
<td>0.88</td>
</tr>
<tr>
<td>2. Acetate-2-14C</td>
<td>1.0</td>
<td>8.43</td>
<td>0.19</td>
<td>1.07</td>
</tr>
<tr>
<td>3. L-Phenylyalanine-U-14C</td>
<td>0.5</td>
<td>2.99</td>
<td>2.64</td>
<td>0</td>
</tr>
<tr>
<td>4. L-Phenylyalanine-U-14C</td>
<td>1.0</td>
<td>8.20</td>
<td>5.92</td>
<td>0.32</td>
</tr>
<tr>
<td>5. DL-Phenylyalanine-3-14C</td>
<td>2.0</td>
<td>20.18</td>
<td>1.36</td>
<td>2.11</td>
</tr>
<tr>
<td>6. L-Tyrosine-U-14C</td>
<td>1.0</td>
<td>38.20</td>
<td>4.93</td>
<td>0.30</td>
</tr>
<tr>
<td>7. DL-Tyrosine-3-14C</td>
<td>2.0</td>
<td>24.20</td>
<td>1.44</td>
<td>2.03</td>
</tr>
</tbody>
</table>

- * 3',6'-Diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid.
- Delution factor from body CoQ9 due to addition of carrier ranged from 5 to 18.
- * 3',6'-Diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid.
- Measured as the bis-dinitrophenylhydrazone.
- Calculated from the activity of the levulinaldehyde.
- * Side chain activity calculated by multiplying molar levulinaldehyde specific activity by 8 plus distribution of acetone.

The extent of incorporation of all precursors tested into total carcass CoQ9 of the rat was of the same order of magnitude, the variation being from 0.005 % with L-phenylalanine-U-14C and L-tyrosine-3-14C to 0.022 % with L-tyrosine-U-14C. L-Tyrosine-U-14C was a considerably better precursor of CoQ9 than L-phenylalanine-U-14C and was slightly better in this series of experiments than acetate-1-14C. DL-Tyrosine-3-14C was only one-fourth as active a precursor of CoQ9 as L-tyrosine-U-14C, although the same differential incorporation rates were not noted for the corresponding radioisomers of phenylalanine. The incorporation of label into total body cholesterol was highest with the acetate-1-14C and acetate-2-14C, intermediate with tyrosine-3-14C, and lowest with phenylalanine-3-14C, phenylalanine-U-14C, and tyrosine-3-14C.

The radioactivity from uniformly labeled phenylalanine and tyrosine appears in the ring fragment and from 0 to 36 % appears in the side chain. This is consistent with the two pathways of incorporation of aromatic amino acid carbon into CoQ, one proceeding by way of homogenisate to acetocetate and then reincorporation via mevalonate into the side chain and the

**TABLE V**

Incorporation of 14C labeled from selected precursors into Coenzyme Q9 and cholesterol in intact, normal, and vitamin A-deficient rats after 3 hours

<table>
<thead>
<tr>
<th>Substrate</th>
<th>No. of animals</th>
<th>Dose per experiment</th>
<th>Yields determined</th>
<th>Amount of crude carcass CoQ isolated</th>
<th>CoQ9 from paper chromatogram</th>
<th>Recrystallized CoQ9</th>
<th>Recrystallized CoQ9 hydroquinone diacetate</th>
<th>Incorporation into CoQ9</th>
<th>Carcass cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acetate-1-14C</td>
<td>10</td>
<td>1.0</td>
<td>104.8</td>
<td>25.2</td>
<td>13.4</td>
<td>4277</td>
<td>4300</td>
<td>4790</td>
<td>0.0150</td>
</tr>
<tr>
<td>2. Acetate-2-14C</td>
<td>10</td>
<td>1.0</td>
<td>46.8</td>
<td>31.1</td>
<td>21.6</td>
<td>3050</td>
<td>1770</td>
<td>1940</td>
<td>0.0050</td>
</tr>
<tr>
<td>3. L-Phenylyalanine-U-14C</td>
<td>10</td>
<td>0.5</td>
<td>77.5</td>
<td>46.0</td>
<td>30.4</td>
<td>618</td>
<td>500</td>
<td>570</td>
<td>0.0053</td>
</tr>
<tr>
<td>4. L-Phenylyalanine-U-14C</td>
<td>10</td>
<td>1.0</td>
<td>37.4</td>
<td>27.5</td>
<td>15.9</td>
<td>2560</td>
<td>2850</td>
<td>2390</td>
<td>0.0050</td>
</tr>
<tr>
<td>5. DL-Phenylyalanine-3-14C</td>
<td>14</td>
<td>2.0</td>
<td>73.3</td>
<td>46.7</td>
<td>32.0</td>
<td>2440</td>
<td>2370</td>
<td>2420</td>
<td>0.0051</td>
</tr>
<tr>
<td>6. L-Tyrosine-U-14C</td>
<td>10</td>
<td>1.0</td>
<td>42.2</td>
<td>36.3</td>
<td>16.1</td>
<td>7440</td>
<td>8550</td>
<td>9050</td>
<td>0.0050</td>
</tr>
<tr>
<td>7. DL-Tyrosine-3-14C</td>
<td>10</td>
<td>2.0</td>
<td>62.2</td>
<td>43.5</td>
<td>29.6</td>
<td>2970</td>
<td>2880</td>
<td>2957</td>
<td>0.0052</td>
</tr>
</tbody>
</table>

* Vitamin A-deficient rats were used throughout except in Experiment 3, in which normal rats were used.
* Total spectrophotometric estimate on nonsaponifiable fractions from liver and carcass.

* Dilution factor from body CoQ9 due to addition of carrier ranged from 5 to 18.
* 3',6'-Diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid.
* Measured as the bis-dinitrophenylhydrazone.
* Calculated from the activity of the levulinaldehyde.
* Side chain activity calculated by multiplying molar levulinaldehyde specific activity by 8 plus distribution of acetone.
other by direct incorporation of a nuclear fragment derived from the ring of phenylalanine and tyrosine into the ring of CoQ9.

Most unexpected were the results obtained with DL-phenylalanine-3-14C and DL-tyrosine-3-14C. These substrates were chosen in order to demonstrate that the β-carbon of these two amino acids was incorporated into the methyl group of the ring fragment as expected from our initial hypothesis (9). The observation that very little radioactivity resided in the ring fragment when these labeled aromatic amino acids were given was not consistent with this hypothesis. It appears from these data that the β-carbon of phenylalanine and tyrosine appears only in the side chain of CoQ9. It eliminates the possibility that the β-carbon of phenylalanine and tyrosine remains attached to the ring fragment in the conversion of the ring of the amino acid to the ring of CoQ9.

The presence or absence of the gastrointestinal tract in these experiments (Table IV) did not in any way influence the rate of incorporation or distribution of radioactivity among the lipids analyzed. The biosynthesis of CoQ9, therefore, is accomplished in the liver itself from metabolites available to it in the absence of the gut. In previous studies (6) it was shown that sterilization of the bowel with antibiotics likewise had no influence on the level or rate of incorporation of intraperitoneally administered precursors.

**DISCUSSION**

In 1959, Gloor and Wiss (14) reported that mevalonate-2-14C was incorporated into the crude CoQ9 of liver from normal and vitamin A-deficient rats. This Q was isolated from liver, chromatographed on alumina and polyethylene powder, and crystallized with carrier CoQ9 to constant specific activity. From this questionable evidence, these workers assumed that the native material in the rat was CoQ9. Subsequently, Gloor and Wiss (26) and Rtiegg et al. (21) reported the isolation of CoQ9 from the livers of vitamin A-deficient rats. We had independently observed that CoQ9 was the main homologue in the normal rat (27) and experiments reported here confirm and extend the previous observation that CoQ9 is biosynthesized to only a limited extent in the liver of the rat from acetate-1-14C. Wiss, Gloor, and Webster (28) found, on the contrary, that mevalonate-2-14C administered either intraperitoneally or orally to rats resulted in 2 hours in the labeling of both hepatic CoQ9 and Q9 in proportion to their concentrations in the liver. Although specific activities were not directly determined, it was inferred from these data that they were the same. In liver slices, we have noted only negligible incorporation of mevalonate-2-14C into CoQ9, whereas incorporation into CoQ9 was excellent (29).

On the other hand, we have observed that CoQ9 isolated from total carcasses of the rats 3 hours after administration of acetate-1-14C is relatively more radioactive than that from the liver alone. It is possible that the results of Wiss, Gloor, and Weber (28) can be explained on the basis of synthesis in the intestine, which has been noted by Lawson et al. (30), and transport to the liver. Since CoQ9 is found in the feces of rats on a CoQ-free diet (6), and since CoQ9 is absorbed and stored in the liver when administered to rats (6, 30), it is probable that a sizable fraction of hepatic CoQ9 in the rat is derived from the microflora of the gut.

It is of some interest that the rat and mouse are the only two mammals which have to date been shown to contain CoQ9 as the predominant homologue. Examination of tissues from beef (3), human (19), horse (31), pig (32), and rabbit (19) have all indicated that CoQ9 is the only homologue present. In plants, both CoQ9 and CoQ10 have been found (33). CoQ10 has been noted in microorganisms (2). The peculiar distribution of CoQ homologues characteristic of any species is no doubt the resultant of the distribution and activity of the enzyme systems which synthesize given elongated isoprenoid alcohol pyrophosphates for condensation with a ring moiety of CoQ9, as well as the capacity of the intestine to absorb other CoQ homologues present in the intestine owing either to diet or to microbiological synthesis.

These results show clearly that acetate is incorporated into the isoprenoid side chain of CoQ9 in the rat and does not contribute any carbon to the aromatic ring system. On the basis of labeling in the isoprenoid side chain only, the calculated incorporation of radioactivity from 14C-acetate into the side chain of 3', 6'-diacetoxy-4', 5'-dimethoxy-2'-methylphenylacetic acid isolated as a ring fragment in the degradations carried out is 5.9% for acetate-1-14C and 3.8% for acetate-2-14C. The experimentally determined values of 5.5% and 2.4%, respectively, are in good agreement with this expectation. Gloor, Schindler, and Wiss (34) found labeled carbon from mevalonate-1-14C exclusively in the side chain of CoQ9 biosynthesized in the rat. These findings are consistent with the repeated observation that the benzene ring cannot be synthesized de novo from small molecules in the animal body and that nutrients containing preformed benzenoid nuclei are essential for the synthesis of such aromatic compounds as epinephrine, norepinephrine, thyroxine, and melanin. On the other hand, the biosynthesis of benzenoid substances via acetyl-CoA and malonyl-CoA is a recognized pathway in certain lower forms, notably fungi (33).

The incorporation of radioactivity from labeled phenylalanine and tyrosine into CoQ9 is unequivocally established by these experiments. Wiss, Gloor, and Weber (28) reported that uniformly labeled phenylalanine orally administered to rats yielded only traces (0.001%) of radioactivity in the non-saponifiable fraction from liver after 2 hours. In a later report (25) these same workers observed that when tyrosine of higher radioactivity was administered per os to rats, 0.2% of the administered dose was found in the non-saponifiable lipids of the liver and 0.0008% of the dose was found in hepatic CoQ9. No degradations of this biosynthesized CoQ9 were reported. In our experiments the extent of incorporation of radioactivity into the total non-saponifiable fraction was 0.6% with acetate-1-14C and acetate-2-14C, 0.1% with 3-phenylalanine-1-14C, and 0.9% with 3-tyrosine-1-14C. The order of magnitude of incorporation of tagged acetate into CoQ9, however, was the same as that observed with the aromatic amino acids. The ratio of specific activity per equivalent weight of isoprenoid carbon for CoQ9-cholesterol was 1.5 to 6.0 for the uniformly labeled aromatic amino acids and 0.5 to 0.6 for acetate-labeled CoQ9. This result conflicts with the observations of Wiss, Gloor, and Weber (25) who found that this ratio was 0.6 in liver lipids after administration of tyrosine-U-14C to rats and 0.4 after mevalonate-2-14C. Of more importance are the results of the chemical degradations of the radioactive CoQ9 biosynthesized in the rat from this battery of precursors. Uniformly labeled phenylalanine and tyrosine contribute carbon to both the nucleus and the side chain of CoQ9 whereas β-labeled phenylalanine and tyrosine contribute carbon only to the side chain. The small amount of radioactivity associated with the ring fragment isolated in these experiments corresponds to that:

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R. E. Olson, unpublished observations.
found with acetate and may be assumed to be in the acetic acid side chain of the substituted phenylacetic acid. These findings eliminate a preformed tolquinone formed from homogentisate by decarboxylation as a precursor of the nuclear fragment in CoQg. Further, it opens up the unprecedented possibility that direct C-methylation of a benzenoid system may occur in animal tissues. In fact, preliminary studies in this laboratory (8) have shown that formate may be incorporated into the ring methyl group of CoQg as well as into the O-methyis, the latter being well established by Glover et al. (36), Rudney and Sugimura (37), and Wiss, Gloor, and Weber (25).

One may well ask what the pathway is for the complete loss of the side chain from phenylalanine in the metabolism of its ring fragment to that of CoQg. The study of possible intermediates in our laboratory has led to the view that benzoate and p-hydroxybenzoate are on this pathway. The sequence is probably as follows:

Phenylalanine → tyrosine → p-hydroxybenzoate → Q nucleus → Q

Since the rate of incorporation of tyrosine into CoQ is approximately 4 times as fast as that of phenylalanine, it is reasonable to believe that tyrosine is more proximal to CoQ than is phenylalanine. The evidence implicating p-hydroxybenzoate as the next major intermediate has been presented in a preliminary communication (8). The evidence available from studies of the incorporation of carboxyl- and ring-labeled benzoate into CoQg is consistent with that obtained from the two radioisomers of tyrosine in that the carboxyl group of benzoate is lost in its conversion to the coenzyme. Although it has been thought that reactions yielding phenols from tyrosine are generally attributed to the microorganisms of the gastrointestinal tract, recent evidence suggests that carbon-by-carbon chain shortening of tyrosine and phenylalanine is a physiological reaction. The conversion of isotopic phenylalanine to benzoate has been demonstrated in animals by Bernhard, Vuilleumier, and Brubacher (38), and in man by Grüner (39). According to the last author, the rate of this conversion is increased in phenylketonuria. The conversion of tyrosine to p-hydroxybenzoate has also been demonstrated in animals by Booth et al. (40). The mechanism of this chain shortening is at present obscure although Oates et al. (41) have demonstrated the conversion of phenylalanine to phenethylenamine in patients with phenylketonuria. It is possible that decarboxylation followed by amine oxidation, typical of the metabolism of catecholamines, followed by α-oxidation of the resulting phenylacetic acid and ultimate decarboxylation is the sequence followed by tyrosine in generating the CoQ ring system. The order of hydroxylations and alkylations (to yield the methyl group and the polyisoprenoid side chain) is, of course, unknown and is a subject for continuing study.

The over-all rate of CoQg biosynthesis in the rat is slow. If one assumes, for the sake of an approximation, that total synthesis de novo of CoQg occurs in its turnover in tissues, it is possible to approximate its rate of turnover from the relative rate of incorporation of acetate-1-14C into the side chain of the coenzyme and into cholesterol, assuming that the isoprenoid pyrophosphates involved in both arise from a common pool. The specific activity of liver CoQg biosynthesized from acetate-1-14C was approximately one-half of that of simultaneously labeled hepatic cholesterol. The liver plasma pool of cholesterol in the rat, which is in isotopic equilibrium in 90 minutes, is approximately 20 times the size of the similar CoQg pool in the rat. There is thus a 40-fold difference in the rate of incorporation of isoprenoid pyrophosphate molecules into the CoQg and cholesterol pools. Lindstedt and Norman (42) estimated from studies of cholesterol turnover and excretion in the young adult rat that 1.5 mg of cholesterol are synthesized per day by rat liver. On this basis, the CoQg biosynthesis by rat liver would be approximately 40 μg per day or 1.5 μg per hour. This very slow metabolic turnover is consistent with the low incorporation rates of isotopes from both radioactive acetate and phenylalanine into total carcass CoQg of from 0.005 to 0.022% of administered isotope in 3 hours.

The contribution of intestinal flora to hepatic CoQ biosynthesis seems negligible in view of almost identical results obtained in intact and enterectomized rats. The suggestion that an unknown aromatic fragment may be derived from the microflora of the gut (30) seems unlikely in view of the satisfactory incorporation of parenteral phenylalanine-1-14C into hepatic CoQg in the enterectomized rat.

SUMMARY

Coenzyme Qg and coenzyme Q10 have been isolated from rat liver and characterized as the free quinones and as the respective coenzyme Q hydroquinone diacetates. Coenzyme Qg is the principal homologue in rat liver, making up 80% of the total coenzyme Q. 14C-Labeled acetate, phenylalanine, and tyrosine are incorporated into total rat coenzyme Qg to the extent of 0.005 to 0.022% of the administered dose. Coenzyme Q10 is labeled much more poorly.

By chemical degradation of the 14C-CoQg hydroquinone diacetate obtained in these experiments the pattern of labeling of the coenzyme was ascertained. With acetate-1-14C and acetate-2-14C, radioactivity was found almost exclusively in levulinic acid derived from the side chain. With uniformly labeled L-phenylalanine-1-14C and L-tyrosine-1-14C, the radioactivity was found predominantly (70 to 88%) in the ring fragment, 3', 6'-diacetoxy-4', 5'-dimethoxy-2'-methylphenylacetic acid. The over-all incorporation of uniformly labeled tyrosine-14C into CoQg was 4 times greater than that observed with uniformly labeled phenylalanine-14C. With D-phenylalanine-3-14C and D-tyrosine-3-14C, however, the incorporation was almost exclusively in the side chain.

These results are interpreted to signify that two distinct pathways exist for the incorporation of radioactivity from uniformly labeled phenylalanine into CoQg in the rat. One pathway involves the conversion of phenylalanine to tyrosine, homogentisate, and acetacetate with reincorporation of the final product into mevalonate which serves as a side chain precursor. The other pathway involves complete loss of the side chain of the phenylalanine acid in the process of generating a nuclear fragment which ultimately becomes the ring system of CoQg. The details of this latter novel pathway are under investigation.

Acknowledgements—We thank Mrs. C. W. Lee Iyengar for technical assistance. We are also indebted to Doctor Marcia RiegI for the care of the animals used in this study and to Doctor Hans Noll of the Department of Microbiology in the School of Medicine for the infrared measurements of coenzyme Qg and

*Note*—It has since been established that p-hydroxyphenylactic and p-hydroxyeinnamic acids are intermediates in the chain-shortening process.
coenzyme Q9. Thanks are finally due to Doctor Karl Folkers of Merck Sharp and Dohme of Railway, New Jersey, and Doctor O. Wiss of Hoffmann-LaRoche, Basle, for generous supplies of authentic coenzyme Q10 and coenzyme Q9.

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Studies on Coenzyme Q: PATTERN OF LABELING IN COENZYME Q9 AFTER ADMINISTRATION OF ISOTOPIC ACETATE AND AROMATIC AMINO ACIDS TO RATS

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