The Origin of Dolichol in the Liver of the Rat

DETERMINATION OF THE DIETARY CONTRIBUTION*

(Received for publication, October 23, 1981)

R. Kennedy Keller†, Eve Jebele, and W. Lee Adair, Jr.‡

From the Department of Biochemistry, University of South Florida College of Medicine, Tampa, Florida 33612

Commercial laboratory rat chow was found to contain 8.5 μg/g of dolichol and 2.1 μg/g of polyprenol; each was 14-18 isoprenes in length. The chain length and relative concentration of rat fecal dolichol and polyprenol were approximately the same as in chow, indicating that there was no preferential absorption of a particular chain length or a particular class of prenols. To quantitate the absorption of these compounds, rats were intubated with either [3H]dolichol or [3H]polyprenol, or, as a control, [3H]cholesterol. After 30 h, the amount of radioactivity accumulated in the liver of the [3H]cholesterol-fed rats averaged 6% of the total administered. In contrast, the amount of radioactivity present in the livers of the [3H]dolichol- or [3H]polyprenol-fed rats was 0.05% of the total. From these data, the maximum hepatic accumulation of the dietary prenols was calculated to be 0.06 μg/30 h. When compared with the values obtained for hepatic de novo synthesis (Adair, W. L., Jr., and Keller, R. K. (1982) J. Biol. Chem. 257, 8989-8996), we conclude that the contribution of the diet to the liver dolichol pool is negligible.

Dolichol is a long chain polyisoprenoid, the phosphorylated form of which participates in the transfer of oligosaccharide chains during N-linked glycoprotein synthesis. There are two possible sources of dolichol in liver, i.e., the diet and de novo synthesis. In order to better understand the metabolism of dolichol, it is necessary that the contribution of each of these sources is quantitated. That the diet could supply a portion of the total liver dolichol pool has previously been discussed by Keenan et al. (1), but has not been rigorously tested. A rat feeding on commercial rodent chow is exposed to material of plant and animal origin; such material could contain appreciable amounts of both polyprenol and dolichol (2). We have analyzed the contribution of these dietary prenols to the total liver dolichol pool in the rat using two experimental approaches. First, we have analyzed the isoprenolog patterns of polyprenol and dolichol found in rat chow and rat feces and compared these patterns with that of rat liver dolichol. In the second approach, we have quantitated the amount of dolichol and polyprenol absorbed from the diet by administering the H-labeled compounds and determining the amount excreted in the feces and accumulated in the liver. From the data obtained, a maximum rate of hepatic absorption of dietary dolichol was calculated. In order to determine the contribution of this rate to the total liver dolichol pool, it was necessary that the rate of endogenous synthesis was also quantitated. This de novo rate is the subject of the following report (3).

EXPERIMENTAL PROCEDURES

β-[4-14C]Sitosterol (58 mCi/mmol) was obtained from Amersham. [1-2H]Cholesterol (44 Ci/mmol) was from New England Nuclear. [1-2H]Dolichol was prepared from pig liver dolichol according to Keenan and Kruczek (4). [1-3H]Polyprenol-16 was prepared from polyprenol-16 by reduction with NaBH₄, according to Keenan and Kruczek was found unsuitable for preparation of the aldehyde from polyprenol since it generated two products when analyzed by HPLC. Studies with model compounds suggested that these products were a-cis and a-trans isomers (6). Dolichol was prepared from pig liver dolichol by the procedure of Keenan and Kruczek (4).

Polyprenol from Pinus elliottii (chain length = 14-18 isoprenes) was prepared according to Burgos et al. (7). Triolein and pig liver dolichol were from Sigma. Polyprenol-16 was from Calbiochem. Silica Gel 60 and Fructose gel 9000 were from Merck. Rodent laboratory chow was from Purina.

Animals—Male rats (175-200 g) of the Sprague-Dawley strain (Harlan Industries, Madison, WI) were used. Rats were fed ad libitum a commercial pelleted diet (Purina) except when specific intubation experiments were performed. Separate metabolic cages were used in order that the feces and urine of individual animals could be collected.

Preparation of Intubation Solution—A solution of the labeled compounds was taken to dryness and dissolved in triolein oil. An emulsion was then prepared according to Borgstrom (8) by sonicating the oil in 3 volumes of an aqueous solution of 7% w/v commercial nonfat dry milk.

Intubation Experiment—Chow was removed during the light cycle (10 h) prior to intubation. At the beginning of the dark cycle, 2 ml of the labeled emulsion was injected into the stomach using a curved feeding cannula and a 5-ml disposable syringe. Eight h after intubation (the time required for the stomach to clear), the commercial lab chow was supplied.

Analysis of Radioactivity in Feces and Liver—Organic extracts of feces and liver were prepared by the method of Folch et al. (9) using a Polytron homogenizer. Aliquots (0.1-5.0 ml) were taken to dryness and analyzed for H and 14C using 20 ml of ACS scintillation cocktail (Amersham). In some cases, aliquots were chromatographed on silica gel thin layers in chloroform. Radioactive regions on the plate were detected using a Packard Model 7200 radiochromatogram scanner.

Quantitation of Dolichols and Polyprenols—Fecal extracts of chow, feces, or liver were prepared using a Polytron homogenizer and filtered over Whatman No. 1 paper. To the filtrates were added [3H]dolichol (10⁵ cpm) to monitor purification and correct for losses.

* This work was supported by Grants GM 25364-03 and CA 28781-02 from the National Institutes of Health and Grant PCM 7804919 from the National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Recipients of Research Career Development Awards from the National Institutes of Health.

‡ In this and the following manuscript (3), we have used the term "polyprenol" to mean a polyprenyl alcohol containing an unsaturated a-isoprene unit. The number of isoprene units in the molecule is indicated following a dash. Thus, α-unsaturated nonadecaprenol would be called polyprenol-19. Derivatives of polyprenol are indicated where appropriate, with the exception of the term dolichol (2,3-dihydroxypolyprenol) which is retained due to its familiarity.
Following treatment with water, according to Folch et al. (9), the lower phases were taken to dryness under reduced pressure. The residues were saponified according to Keller et al. (10) and extracted several times with light petroleum. These extracts were taken to dryness, dissolved in chloroform/methanol (2:1), and chromatographed on Fractogel 6000 columns (0.9 × 30 cm) in chloroform/methanol (2:1). The radioactive fractions were pooled and subjected to normal-phase HPLC as described below. Quantitation was achieved by integration of the peaks produced by UV monitoring of the HPLC eluant and comparison with standards of known concentration.

**Analytical Techniques**—HPLC was carried out using a Lab Data Control instrument with on-line UV detection (210 nm) and integration. Normal-phase HPLC was achieved on a 25-cm Partisil-5 column (Whatman) using hexane/ethyl ether (90:10). Reversed-phase HPLC was carried out (11) in methanol/isopropyl alcohol (50:50) on a 25-cm Partisil-5 ODS column (Whatman). All chromatography was performed at room temperature at a flow rate of 2.0 ml/min. TLC was carried out on plastic backed Silica Gel 60 plates (Merck). Lipids were detected using iodine vapor.

**RESULTS**

Standardization of Elution of Polyprenols and Dolichols on Normal-Phase and Reversed-Phase HPLC—Since rat chow is derived from material of plant and animal origin, it was expected to contain both high molecular weight polyprenol and dolichol (2). Polyprenol-19 and dolichol-19 were thus used to determine the positions of elution of these compounds from HPLC. It was found that, in the normal-phase mode, polyprenol-19 eluted earlier than dolichol-19 (Fig. 1). Also, isoprenologs of a given class (polyprenol or dolichol) were found to elute in the order high Mr → low Mr (6). However, the separation was minimal: the isoprenologs had to differ by at least 4 isoprene residues before significant resolution was observed. Reversed-phase HPLC separates prenols primarily on the basis of chain length: dolichol or polyprenol was resolved into its various isoprenologs with the lower molecular weight compounds eluting first. Plots of log elution volume versus isoprene number were nearly linear (Fig. 2) and served as standard curves to determine the number of isoprenes in an unknown. Interestingly, polyprenol and dolichol isoprenologs of the same chain length did not co-migrate, the polyprenol eluting slightly earlier.

Analysis of Prenols in Rat Chow—When the high molecular weight prenol fraction from rat chow, isolated through the Fractogel 6000 step, was analyzed by normal-phase HPLC, two major peaks were observed (Fig. 3). The first peak eluted at the same position as polyprenol from Pinus elliottii (14-18 isoprenes), while the second peak eluted slightly after the position of pig liver dolichol (17-20 isoprenes). To further investigate the nature of the two components, each peak was pooled and subjected to oxidation according to Keenan and Kruczek (4). When the resulting aldehyde products were analyzed by TLC (in chloroform) on plates impregnated with fluorescent dye, peak 1 yielded a band which co-migrated with...
Dietary Contribution to the Liver Dolichol Pool

polyrenal-16 ($R_p = 0.8$) and showed strong absorbance of UV light. The peak II product, on the other hand, co-migrated with dolichal ($R_p = 0.9$) and was UV-negative. These results indicated that the oxidation products of peaks I and II were $\alpha,\beta$-unsaturated and $\alpha,\beta$-saturated aldehydes, respectively. It was concluded, therefore, that peak I was a polyrenol and peak II was a dolichol.

In order to determine the chain lengths of peaks I and II, each was analyzed by reversed-phase HPLC. The pattern generated by peak I was very similar to that observed with the standard polyrenol from *Pinus elliotii* (Fig. 4), indicating a chain length of 14-18 isoprenes. Peak II yielded a pattern indicative of dolichol isoprenologs also of 14-18 isoprenes in length (Fig. 5).

The data obtained thus far indicated that rat chow contained polyrenol and dolichol and that both types of alcohols contained 14-18 isoprene units. Quantitation of these two components, carried out as described under “Experimental Procedures,” indicated that the chow contained polyrenol and dolichol in the ratio 1:4 and that the sum of these two components equaled $11.1 \pm 2.4 \mu$g/g ($n = 6$).

**Analysis of Dolichols in Rat Liver and Feces**—When the material from Fractogel 6000 chromatography of rat liver nonsaponifiable lipids was subjected to HPLC in the normal-phase mode, a single peak was observed in the region where standard pig liver dolichol eluted (data not shown). Quantitation of this peak indicated that rat liver contained 20 $\mu$g of dolichol/g of liver. This value agreed well with those reported by Wenstrom and Hamilton (17.1 $\mu$g/g) (12), Tavares et al. (21.5 $\mu$g/g) (13), and Tavares et al. (22.6 $\mu$g/g) (14). Reversed-phase analysis of the rat liver dolichol sample indicated that the major species was dolichol-18. These results are in accordance with the findings of other investigators concerning rat liver dolichol (12, 14). From these data, it is clear that in order for the diet to contribute to the liver dolichol pool, there must be a specific system in the rat which either absorbs, elongates, and saturates polyrenol or selectively absorbs and incorporates the longer chain isoprenologs of dolichol. A third alternative, the uptake of the lower chain isoprenologs of dolichol, their desaturation, elongation, and resaturation at the $\alpha$-isoprene, was deemed remote.

In order to distinguish between the first two possibilities, prenols were isolated from rat feces and analyzed by normal-phase HPLC. The pattern obtained was indistinguishable from that found for chow prenols (data not shown). Quantitation suggested that either polyrenol and dolichol were not absorbed or that they were absorbed in equal proportions. Reversed-phase HPLC analysis of peak I and peak II obtained from the normal-phase HPLC run gave polyrenol and dolichol isoprenolog patterns, respectively, which were indistinguishable from those obtained from the chow sample. These results appeared to rule out the possibility that the longer chain isoprenologs of either type of prenol were preferentially absorbed by the rat. Quantitation of the prenols in rat feces indicated that the rat secretes approximately the same amount of total prenol as is consumed on a daily basis. Since the rat ingests approximately 15 g of chow per day, then, based on the data given above, the total daily consumption is on the order of 125 $\mu$g of dolichol and 31 $\mu$g of polyrenol. Thus, absorption of a small percentage ($\leq 5\%$) of the dietary polyrenol or longer chain isoprenologs of dolichol could result in a substantial daily contribution to the total liver dolichol. Such a small degree of uptake may have escaped detection in our comparative analysis of the chow and feces.
Balance Studies with [3H]Polyrenols—To investigate the possibility of a small amount of absorption contributing to the total liver dolichol, the following feeding experiment was performed. Rats were intubated with an emulsion containing either [3H]dolichol, [3H]polyprenol-16, or [3H]cholesterol. [14C]Sitosterol, a cholesterol analog which is poorly absorbed (13), was added to correct for recoveries. Rats were killed after 30 or 48 h and the accumulated feces were extracted with chloroform/methanol (2:1) and analysed for 3H/14C.

The ratios of 3H/14C in the fecal extracts of the animals fed [3H]dolichol and [3H]polyprenol were very similar to the starting ratios of the intubation suspensions (Table I), indicating that little if any dolichol was absorbed. In contrast, the ratio from the [3H]cholesterol-fed animals exhibited a significant decrease indicating, as expected, that there was substantial absorption of this lipid. TLC in chloroform of aliquots of the fecal extracts indicated that about one-half of the [3H]dolichol was excreted unchanged and one-half as a less polar species. Following saponification of the fecal extract, all of the radioactive material was found to co-migrate with dolichol. This finding suggested that some of the dolichol had been esterified during passage through the alimentary canal. Since a large bolus of triolein was administered with the dolichol, it is possible that a chemical transacylation reaction took place. However, enzymatic acylation of dolichol, reported by Keenan and Kruczek (16) to occur in rat liver microsomes, could also have occurred. TLC of the fecal extract from a [3H]polyprenol-fed rat indicated a major peak of starting material, a faster moving peak (presumably an ester), and several slower moving minor species. The nature of these compounds was not investigated further.

The above balance studies indicated that little if any dolichol or polyprenol was absorbed by the rat and thus confirmed the analysis on chow and feces described above. In order to quantitate the percentage of administered prenols accumulated by the liver, Folch extracts were prepared and analyzed for 3H counts/min (Table I). Thirty h after intubation, an average of 0.046% of the total 3H counts/min was present in the liver of the [3H]dolichol-fed rats. Approximately the same amount (average = 0.053%) of the total 3H counts/min was present in the liver of the [3H]polyprenol-fed rats. By contrast, an average of 5.8% of the total 3H counts/min was present in the [3H]cholesterol-fed rats. At 48 h, a lower percentage of accumulated radioactivity was found in livers from rats intubated with either of the three labeled compounds (Table I).

**DISCUSSION**

Because of their ubiquitous nature, polyprenol and dolichol probably occur in the diet of most mammals, including man. Therefore, we expected that considerable amounts of ingested prenols might be absorbed, transported to the liver or other tissues, and converted to dolichyl phosphate for use in glycoprotein biosynthesis. The results presented in the present

---

**Table I**

| Expt | Rat No. | Administered | [3H] cpm | [14C] cpm | [3H]/[14C] | [3H] cpm | [14C] cpm | [3H]/[14C] | Excreted | Ratio Admin. | [3H] cpm | Excreted | Total | [3H] cpm in Liver | [3H]% in Liver |
|------|---------|-------------|---------|---------|----------|---------|---------|----------|---------|------------|---------|---------|-------|-----------------|-------------|
| I (30 h) | 1 | [3H]-dolichol + [14C]-sitosterol | 16.0 | 0.072 | 222 | 9.00 | 0.041 | 220 | 0.09 | 8.1 | 0.041 | I | 16.0 | 0.072 | 222 | 9.00 | 0.041 | 220 | 0.09 | 8.1 |
| | 2 | [3H]-dolichol + [14C]-sitosterol | 16.0 | 0.072 | 222 | 10.60 | 0.042 | 252 | 1.13 | 6.0 | 0.038 | I | 16.0 | 0.072 | 222 | 10.60 | 0.042 | 252 | 1.13 | 6.0 |
| | 3 | [3H]-polyprenol + [14C]-sitosterol | 15.6 | 0.068 | 223 | 12.50 | 0.050 | 250 | 1.09 | 9.6 | 0.063 | I | 15.6 | 0.068 | 223 | 12.50 | 0.050 | 250 | 1.09 | 9.6 |
| | 4 | [3H]-polyprenol + [14C]-sitosterol | 15.6 | 0.068 | 223 | 9.60 | 0.039 | 246 | 1.07 | 7.0 | 0.045 | I | 15.6 | 0.068 | 223 | 9.60 | 0.039 | 246 | 1.07 | 7.0 |
| | 5 | [3H]-cholesterol + [14C]-sitosterol | 8.5 | 0.072 | 118 | 3.08 | 0.037 | 83 | 0.70 | 0.70 | 4.0 | I | 8.5 | 0.072 | 118 | 3.08 | 0.037 | 83 | 0.70 | 0.70 |
| | 6 | [3H]-cholesterol + [14C]-sitosterol | 8.5 | 0.072 | 118 | 1.14 | 0.025 | 46 | 0.39 | 6.39 | 7.5 | I | 8.5 | 0.072 | 118 | 1.14 | 0.025 | 46 | 0.39 | 6.39 |

---

I mg of prenol was also added as carrier.
study appear to rule out such a possibility. Thus, an average of only 0.05% of ingested polyprenol or dolichol was found in the liver 30 h after intubation with a suspension of the labeled compound. Because of the low amount of radioactivity in the liver extracts, it was not possible to identify the labeled compounds as dolichol or polyprenol. It is unlikely that our $^3$H-labeled compounds are greater than 99.95% pure, and it is thus possible that a substantial amount (up to 100%) of the radioactivity absorbed is due to contamination in the original sample. Since the rat consumes about 125 μg of dolichol and 31 μg of polyprenol per day, it follows that the maximum amount of dolichol incorporated into the liver is probably less than 0.06 μg. Studies reported in the following manuscript indicate that the de novo rate of hepatic dolichyl phosphate (and presumably dolichol) synthesis is 1.7-2.6 nmol (2.2-3.4 μg, assuming $M_r = 1300$ for dolichol) per day. Therefore, the contribution of the diet to the pool of dolichol or dolichyl phosphate in rat liver must be negligible.

The metabolism of dolichol, at least in the rat, appears to be significantly different from that of cholesterol in several respects. First, the absorption of cholesterol, while dependent on many factors, has been observed to be substantial under most conditions so far investigated (8, 17). Second, the de novo synthesis of cholesterol by the liver is acutely affected by cholesterol feeding (18, 19). Such a regulatory mechanism is unlikely for dolichol biosynthesis, in view of the meager quantity of dolichol absorbed. Whether the liver synthesizes dolichol exclusively for its own use or, in addition, for export to other tissues, is unknown. Keenan et al. (1) found that labeled dolichol administered intravenously appeared first in the high density lipoprotein fraction and later accumulated primarily in the liver; no other tissue accumulated more than 5% of the total dose. This finding, coupled with our (20) report that dolichol levels in avian blood were less than 0.4 μM (cholesterol is 3.7 mM; retinol, 1-2.5 μM), would argue against the export of dolichol by the liver to other tissues. In addition, several tissues have been shown to synthesize dolichol compounds, including liver (10, 12, 14), brain (21), heart (22), testes (12, 23), and spleen (24). Thus, it appears that tissues carrying out glycoprotein synthesis may rely exclusively on their own de novo pathway for producing dolichol.