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DIFFERENT TISSUE CONCENTRATIONS AND RATES OF SYNTHESIS OF DOLICHOL, DOLICHYL ACYL ESTERS, AND DOLICHYL PHOSPHATE IN MOUSE TESTES AND PREPUTIAL GLANDS

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SUMMARY. The rates of synthesis and tissue concentrations of dolichol derivatives differ markedly in testes and preputial glands from adult C57BL/6J mice. In testes, the rates of free dolichol, dolichyl acyl esters, and dolichyl phosphate synthesis were approximately 5,000, 4,000 and 2,000 dpm/3h/g, respectively. Comparable rates for preputial glands were 12,000, 60,000 and 0 dpm/3h/g. Thus in testes, dolichyl phosphate represented 15-20% of total dolichol synthesis, whereas no de novo dolichyl phosphate synthesis was detected in preputial glands. In testes, free dolichol was the predominant derivative synthesized; in preputial glands, 80% of dolichol synthesized was esterified to fatty acids. The concentration of total dolichol (free alcohol plus acyl esters) was 160 μg/g tissue in testes and 1270 μg/g tissue in preputial glands. In both tissues, 85-90% of dolichol was esterified to fatty acids, and no dolichyl phosphate was detected.

INTRODUCTION. Although dolichol is present in cells as the free alcohol, as dolichyl phosphate, and esterified to fatty acids, the only form of dolichol for which a function has been described is dolichyl phosphate, the carrier of sugars during assembly of N-linked glycoproteins. There is little information available about the rates of synthesis of these dolichol derivatives in animal tissues. Spermatogenic mouse testes synthesized dolichol + dolichyl acyl esters at a higher rate than did mouse cell cultures and liver (1-4). De novo synthesis of dolichyl phosphate has been reported to occur in two differentiating tissues, developing sea urchin embryos (5) and erythropoietic spleens from phenylhydrazine-treated mice (6). The synthesis of dolichyl phosphate was measured in rat liver slices and in isolated hepatocytes (7).

The synthesis of dolichol, dolichyl acyl esters, and dolichyl phosphate has been measured in two mouse tissues in which continuous cell differentiation occurs, the testes and the preputial gland. The preputial glands, located under the skin in the lower abdomen of the mouse, are holocrine organs analogous to the sebaceous gland. Basal cells of the gland divide and differentiate to

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produce lipid, which is released as the glandular secretion when the mature cells lyse. It is reported here that the mouse preputial gland synthesized dolichyl acyl esters at a much higher rate per gram tissue than has been reported for other mouse tissues, and that no de novo dolichyl phosphate synthesis was detected. In contrast, the predominant dolichol derivative synthesized by testes was the free alcohol, and significant dolichyl phosphate synthesis was measured. The relative and absolute synthetic rates of free dolichol, dolichyl acyl esters and dolichyl phosphate, and the ratio of dolichol to cholesterol synthesis, were markedly different in preputial glands and testes. Values for the tissue concentrations of the dolichol derivatives are also presented here.

MATERIALS AND METHODS. The following compounds were obtained from New England Nuclear Corp.: [1-^{14}C]acetic acid, sodium salt (57 Ci/mol), [1,2-^{3}H]cholesterol (40 to 60 Ci/mmol), [1-^{3}H]dolichol (12.5 Ci/mmol). HPLC solvents (Fisher) were used for high pressure liquid chromatography. Wheat germ acid phosphatase (0.36 units/mg) was from Sigma.

Mice. 8-week-old male C57BL/6J mice were obtained from the Animal Resources Department of the Jackson Laboratory.

Incubation of tissues. Animals were killed by cervical dislocation. Preputial glands were removed and carefully teased apart; testes were decapsulated and teased apart. Glands or testes from 5 mice were incubated at 37°C for 3 hours in 5 ml of Krebs-Ringer buffer (1) containing 200 μCi of [^{14}C]acetate. Before incubation, the flasks were flushed with air: CO_2(95:5).

Lipid extractions. To obtain dolichol, washed tissues were extracted by homogenizing in water and extracting with 20 vol of chloroform-methanol (2:1, v/v) containing butylated hydroxytoluene as antioxidant. Extracts were washed with 0.2 vol of 0.03% $MgCl_2$. To obtain dolichol from dolichyl acyl esters, tissues were saponified at 80°C for 2 1/2 hours in 30% ethanolic KOH containing pyrogallol and butylated hydroxytoluene, and extracted as described (2). One-half of the nonsaponifiable lipid sample was treated with wheat germ acid phosphatase to obtain dolichol from dolichyl phosphate, using conditions as described (5). These extraction methods were also used in determining the levels of dolichol in freshly excised tissues without incubation. [^{3}H]cholesterol and pig liver [^{3}H]dolichol standards were added to all tissues.

High pressure liquid chromatography. The μBondapak C18 reverse-phase column system (Waters Assoc., Milford, MA) was described (2). Two solvent systems were used: HPLC System A, 1% aqueous methanol for 30 min. then a linear gradient over 25 min. to a final concentration of 35% methylene chloride in the original solvent (1% aqueous methanol), flow rate 2 ml/min; System B, concave gradient No. 10 (Waters Assoc. Model 660 Solvent Programmer) from 100% methanol to 100% methylene chloride over 48 min., flow rate 2 ml/min. For mass measurements, the dolichol-containing fraction obtained from system B was then rechromatographed on a μBondapak C18 reverse-phase column, solvent system (system C) 1:1 methanol:isopropanol, flow rate 2 ml/min, column temperature 55°C, as described (8). HPLC system C separated dolichol into individual isoprenologues.

Measurement of [^{14}C]acetate incorporation into cholesterol and dolichol. The dolichol fraction collected by HPLC System B was acetylated and chromatographed on thin layer silica gel plates with toluene as solvent; each plate was divided into 1 cm bands, and ^{14}C and ^{3}H in acetylated dolichol were determined. Overlap of ^{3}H into the ^{14}C counting channel was less than 0.05%. Radiolabeled cholesterol was determined as digitonin-precipitable sterol (9). For reasons of brevity, acetate incorporation into dolichol and cholesterol is hereafter referred to as synthesis of the respective compounds.

Mass measurements of dolichol. Lipid samples were prepared as described above for mass measurements of dolichol. [^{3}H]dolichol standard was added to tissue samples prior to extraction, and ^{3}H in dolichol samples from HPLC was counted to correct for recovery. The non-saponifiable lipid samples were separated by HPLC systems B and C, and the concentration of dolichol was determined by absorption at 210 nm (ISCO absorbance monitor model 1840 and Spectra-Physics SP4100 computing integrator) using described procedures (8).

RESULTS AND DISCUSSION. Dolichols of several chain lengths were synthesized from [^{14}C]acetate by mouse preputial glands, as shown in Fig. 1. The major isoprenologue in standard ^{3}H pig liver dolichol contains 19 isoprenoid units (10), and the [^{14}C]dolichol synthesized by preputial glands contains primarily 18, 19, and 20 isoprenoid units. It was shown previously that testes dolichols were slightly more polar than pig liver dolichols of corresponding chain length (1). Preputial gland dolichols also eluted slightly ahead of the corresponding

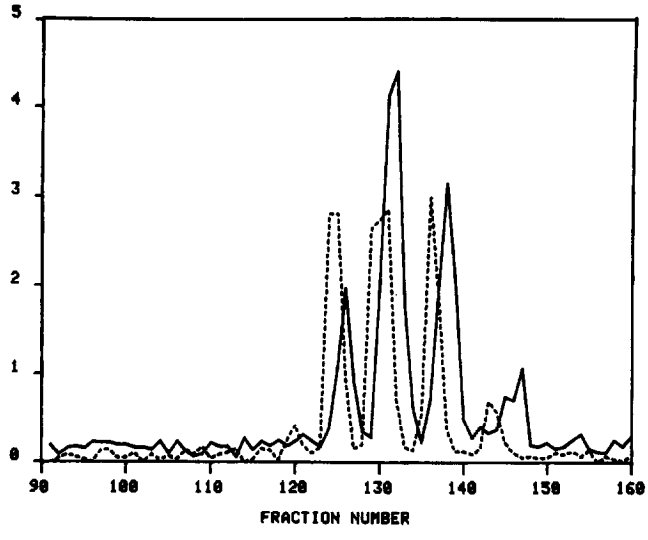

Figure 1 High pressure liquid chromatography chromatograms of acetylated dolichol synthesized from [^{14}C]acetate by C57BL/6J mouse preputial glands. Preputial glands from 5 mice were incubated with [^{14}C]acetate, saponified, extracted, and the dolichol sample was obtained using HPLC System B. Acetylated dolichol was eluted from thin-layer chromatography plates and chromatographed using HPLC system A; 1 ml samples were collected and ^{3}H and ^{14}C were assayed as described in the text. The dotted line represents ^{3}H pig liver dolichol standard (Y-axis = dpm × 0.5 × 10^{-4}); the solid line represents ^{14}C lipids synthesized by preputial glands (Y-axis = dpm × 10^{-2}).

TABLE I. INCORPORATION OF [^{14}C]ACETATE INTO DOLICHOL DERIVATIVES BY MOUSE

	Testes	Preputial gland
	dpm/3h/gram tissue	
Dolichol	5,408 ± 1161(4)	11,631 ± 1944(3)
Dolichol + dolichyl acyl esters	9,656 ± 1288(4)	58,908 ± 7063(3)
Dolichol + dolichyl acyl esters + dolichyl phosphate	11,579 ± 1899(4)	58,763 ± 4928(3)
Dolichyl phosphate, % of total	17	0
Dolichol/cholesterol × 100, %	2.5	6.0

The incorporation of [^{14}C]acetate into dolichol, dolichyl acyl esters, dolichyl phosphate, and cholesterol was measured as described in the text. Results are expressed as dpm/3h/g tissue ± SD. The number of separate experiments is in parentheses.

pig liver dolichols. The increased polarity may be due to the presence of an unsaturated α-isoprene unit in the newly synthesized molecule (1).

The rates of synthesis of dolichol derivatives differed in the testes and preputial glands. As shown in Table I, no synthesis of dolichyl phosphate was detected in the preputial gland, whereas in testes dolichyl phosphate accounted for approximately 17% of total dolichol synthesis. In testes, approximately 50% of total dolichol was synthesized as the free alcohol, and approximately 35% of newly synthesized dolichol was esterified to fatty acids. Eighty percent of the dolichol newly synthesized by preputial glands was esterified to fatty acids and only 20% was present as the free alcohol. Total dolichol synthesis was 2.5% of the rate of cholesterol synthesis in testes and 6% in preputial glands.

The concentration of dolichol derivatives in testes and preputial glands is presented in Table II. In testes, 89% of the dolichol was esterified to fatty acids and, in preputial glands, dolichyl acyl esters represented 84% of total dolichol. When the saponified lipid samples were treated with wheat germ acid phosphatase, no increase in the concentration of free dolichol was detected. This indicates that dolichyl phosphate levels are negligible in comparison with dolichyl acyl ester concentration in testes and preputial glands. To our knowledge, this is the first report of the levels of dolichol derivatives in these mouse tissues. Rupar and Carroll (11) found 3226 μg

TABLE II. CONCENTRATIONS OF DOLICHOL DERIVATIVES IN TESTES AND PREPUTIAL GLANDS FROM C57BL/6J MICE

	Testes	Preputial glands
	μg/gram tissue ± SD	
Dolichol	17.0 ± 2.7(3)*	203 ± 46(4)
Dolichol + dolichyl acyl esters	159.5 ± 9.5(5)	1318 ± 141(2)
Dolichol + dolichyl acyl esters + dolichyl phosphate	150.4 ± 16.1(4)	1227 ± 290(2)
% Dolichol esterified to fatty acids	89%	84%

Lipids were extracted as described in the methods section, and dolichol in each sample was obtained by HPLC system B. This dolichol fraction was quantitated by UV absorbance, using HPLC system C.

*Number of tissue samples.

dolichol/g wet weight of human testes, but in striking contrast to the results reported here, only 3% of human testes dolichol was esterified to fatty acids. However, in that study (11) there was no mention of correcting for loss of dolichol during extraction and column chromatography. According to a few reports in the literature, the concentration of dolichol in the same tissues from different mammals varies considerably. Burgos et al. (12) reported that pig liver contained 60 μg dolichol + dolichyl acyl esters/g tissue, whereas human liver contained 1226 μg free dolichol/g tissue and 136 μg dolichyl acyl esters/g tissue. Keller and Adair (13) found that pig liver contained 129 μg of dolichol + dolichyl acyl esters/g tissue. Human pituitary contained 1400 μg free dolichol/g tissue and beef pituitary contained 97 μg free dolichol/g tissue; in both of these tissues, 25% or less of the dolichol was esterified to fatty acids (14).

The only reports of the levels of dolichyl phosphate in tissues are by Keller et al. (15), who found 3.9 μg dolichyl phosphate/g rat liver, Carson and Lennarz (5) who reported that sea urchin embryos contain 0.1 - 0.6 nmoles dolichyl phosphate/mg protein during embryogenesis, and Potter and Kandutsch (6) who found that dolichyl phosphate levels in mouse spleens varied from 11.2 - 41.4 μg/g tissue during phenylhydrazine-induced erythropoiesis. Measurements of the levels of dolichyl phosphate in other tissues is an impor-

tant aspect of learning about the regulation of the dolichol pathway. Two of the studies cited above (5,6) showed that dolichyl phosphate levels can change during differentiation; this observation is interesting in view of studies showing that dolichyl phosphate levels are rate-limiting for protein glycosylation (16-19).

The preputial gland has been utilized extensively in studies of sterol and ether lipid synthesis (20-23), and has been used for investigating the hormonal control of cell division and lipid synthesis in sebaceous-type glands (24,25). The primary secretion product of the gland is lipid, and hence the high rate of dolichol synthesis in the gland perhaps cannot be explained simply on the basis of a need for glycoprotein synthesis, as has been shown for developing sea urchin embryos (5). However, in sea urchin embryos there was a high rate of dolichyl phosphate synthesis which correlated with N-linked glycoprotein assembly (5), whereas no de novo synthesis of dolichyl phosphate was detected in the preputial gland. The amount of cellular protein increased less than 2-fold during differentiation of preputial gland cells whereas the amount of cellular lipid increased more than 15-fold (26,27). Future experiments may indicate whether the apparent difference in the rate of dolichyl phosphate synthesis in these tissues may be due to the presence of phosphatase activity in the preputial gland. In addition, it may be possible to obtain information about the role(s) of dolichyl acyl esters in these organs, and about the correlation between rates of dolichyl phosphate synthesis and glycoprotein assembly.

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