Elevated Dolichol Synthesis in Mouse Testes during Spermatogenesis*

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The synthesis in mouse testes of the isoprenoid lipid dolichol, the phosphate esters of which are involved in glycoprotein assembly, was investigated. Because the pathways for dolichol and cholesterol branch from a common sequence of reactions, comparisons of the rates of synthesis of these two lipids can reveal specific alterations in the flow of metabolites along the branch leading to dolichol. Our results showed that the ratio of dolichol to sterol synthesis in testes was from 26- to 72-fold higher than any ratios we have measured in murine liver or cell cultures. In contrast, testes deficient in spermatogenic cells, from x-irradiated mice, or from mice with mutant W/W" or at/at genotypes, synthesized normal amounts of cholesterol, but much less dolichol. Thus, the high rate of dolichol synthesis in normal testes appeared to be associated with one or more of the spermatogenic cell types. It was also observed that newly biosynthesized testicular dolichols are slightly more polar than the pig liver dolichols used as standards. Possible modifications of the dolichol structures that might account for the increased polarity are discussed.

Dolichols are long chain polyisoprenes comprising 16 to 22 isoprene units which, in the form of dolichyl pyrophosphate and dolichyl phosphate, function as lipid carriers for saccharyl residues in the assembly of N-glycosidically linked proteins (1-3). This function of dolichol has been considered to be essential. However, there exists little information regarding the biosynthesis of dolichol, even though it has many biosynthetic enzymes in common with the widely studied pathway of cholesterol synthesis. The incorporation of radioactively labeled mevalonate into dolichol in vivo has been demonstrated in rat liver (4, 5), and in rabbit and pig liver (6). In the latter study (6), the livers of intact animals incorporated [2,4-14C]mevalonate into dolichol at 0.09 to 0.2% of the rate of incorporation into the total unsaponifiable lipid fraction. The incorporation of mevalonate into dolichylpyrophosphoryl oligosaccharide has also been demonstrated (7, 8). Recently, we demonstrated that various mammalian cell cultures, growing in lipid-free media, incorporated [14C]acetate into dolichols and into cholesterol by mouse testes. The data showed that the rate of dolichol synthesis in testes was high by comparison with liver. Evidence from studies with testes deficient in spermatogenic cells indicated that one or more of these cell types was responsible for the high rate of dolichol synthesis.

MATERIALS AND METHODS

Animals—Homozygous at/at (atrichosis) males were obtained from the Mouse Mutant Stocks Center of The Jackson Laboratory from a balanced breeding colony (inbred for 19 generations) where at and eb (eye-blebs) are kept segregating in repulsion (at/+ and eb)1. The controls were normal littermates (at/+ and eb). The controls were normal littermates (at/+ and eb) and these mice were used at approximately 12 weeks of age. Mice bearing the genotype W/W" were F1 hybrids of the inbred strains C57BL/6J-W/W" and WB/Re-W/+ maintained by Dr. E. S. Russell of The Jackson Laboratory. The controls had the genotype +/+ at the W locus and these mice were used at 7 to 8 months of age. C57BL/6By male mice, from the Animal Resources Department of The Jackson Laboratory, were used at 9 weeks of age for the experiments involving x-irradiation of testes. A General Electric Maxitron 250 containing a copper (0.5 mm)/aluminum (1 mm) filter was used to generate the x-rays. The abdomens of mice were shielded while their testes were exposed to x-ray doses of 300 R, followed by 100 R after 4 days.

High Pressure Liquid Chromatography—The HPLC1 systems used in this study, employing a Spherapak C18, reverse-phase column (Waters Associates), have been previously described (9). The two solvent systems used are: HPLC System A, 1% aqueous methanol for 32 min and then a linear gradient over 25 min to a final concentration of 50% methanol in the original solvent (1% aqueous methanol); HPLC System B, concave gradient No. 10 (Waters Associates) model 660 solvent programmer) from 100% methanol to 100% meth-

1 The abbreviation used is: HPLC, high pressure liquid chromatography.
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RESULTS

Chain Lengths of Mouse Testes Dolichols—As shown in Fig. 1, dolichols of varying chain length were resolved by HPLC System A. Since the predominant isoprenologue in the pig liver 3H-dolichols which served as standards contains 19 isoprenoid units (13), the major dolichol synthesized in the testes appeared to contain 18 isoprenoid units. This result is similar to that obtained with various murine cell cultures in which the predominant isoprenologue of dolichol also contained 18 isoprenoid units (9). However, the 14C-dolichol bands synthesized by the testes from acetate in Fig. 1 are not exactly coincident with the corresponding standard 3H-dolichol bands, but consistently precede them by one fraction.

In order to determine whether the apparent displacement of the peaks of biosynthesized dolichols relative to the pig liver standard could be due to interference by extraneous 14C-lipids eluted with the dolichols, two further tests were carried

![HPLC chromatograms (HPLC System A) of dolichols synthesized in mature testes from 14C-]acetate (a and b) or from 14C]mevalonate (c and d) and chromatographed as the free alcohols (a and c) or as the acetates (b and d). Five flasks, each with eight testes, 200 μCi of 14Cacetate (3.5 μmol) and 5 ml of buffer, were incubated for 3 h and then combined or 10 testes were incubated with 50 μCi of 2-14C]mevalonolactone (1.1 μmol) in 5 ml of buffer for 5 h. Preparation and chromatography of the free dolichols is described in the text. The acetylated dolichols were prepared as follows: aliquots of the nonsoapifiable lipids were chromatographed using HPLC System B, the dolichol-containing fractions were collected, acetylated, run on TLC using toluene as the solvent, and finally eluted from the TLC plate with CHCl3/CH3OH (2:1). Aliquots of the above lipid preparations were chromatographed using HPLC System A. Fractions (1 ml) were collected and assayed for 3H and 14C as described in the text. The solid lines represent 14C-lipids synthesized by the testes. The dotted lines represent reference 3H]dolichol from pig liver.

Table I

Synthesis of dolichol and cholesterol from 14Cacetate in testes and livers of normal and mutant mice

The incorporation of 14Cacetate into dolichol and cholesterol was measured as described in the text. The mouse strains carrying the indicated genotypes are identified in the text. The numbers in parentheses represent the number of testes minced in the pool which was used for each assay. For each determination involving liver, 250 mg of liver slices were taken from each of four mice, pooled, and incubated as described in the text.

| Genotype | Testes | Liver |
|----------|--------|-------|
|          | Weight | Dolichol | Cholesterol | Dolichol/chol- | Dolichol | Cholesterol | Dolichol/chol- |
|          | mg     | dpm/testis/3 h | % | dpm/mg/3 h | % |
| +/+      | 130 (4) | 1,884 | 80,124 | 2.36 | 0.38 | 1,417 | 0.05 |
| W/W      | 14 (12) | 26 | 105,276 | 0.02 | 0.74 | 3,833 | 0.02 |
| +/?      | 99 (4) | 1,520 | 82,539 | 1.96 | 1.56 | 3,077 | 0.05 |
| 4d/at    | 18 (16) | 17 | 37,629 | 0.04 | 0.74 | 2,151 | 0.03 |

* Average wet weight per testis.
Dolichol Synthesis in Mouse Testes

The data in Table I show that there is a high rate of dolichol synthesis in normal mouse testes. Minces of mouse testes were incubated with \[^{14}C\]mevalonate which was expected to be incorporated predominantly into isoprenoid lipids. The free and acetylated \(^{14}C\)-dolichols were then assayed by HPLC exactly as were the dolichols synthesized from acetate (Fig. 1, c and d). The results of these experiments established the identity of the \(^{14}C\)-labeled lipids as long chain polylsoprenols (i.e. dolichol species) and confirmed the slight increase in the polarity of these compounds relative to those in the tritium-labeled pig liver, standard preparation.

**Dolichol Synthesis in Mouse Testes during Spermatogenesis**—Human testes have been shown to contain high concentrations of dolichol by comparison with the concentrations in other tissues (12). The data in Table I show that there is a high rate of dolichol synthesis in normal mouse testes. Minces of normal mouse testes incorporated \[^{14}C\]acetate into dolichol at a rate 1.96 to 2.35% of the rate of incorporation into cholesterol (Table I). These ratios of dolichol to sterol synthesis represent an average increase from 20- to 72-fold over ratios we reported previously for various cultured cells (9), and an average increase of 43-fold over the ratios of 0.05% measured in normal mouse liver (Table I). During 3-h incubations, the average rate of dolichol synthesis from \[^{14}C\]acetate/mg of normal liver was 1.12 dpm, whereas the corresponding average rate for normal testes was 15.4 dpm/mg (derived from data in Table I) which represents an increase of 14-fold in testes.

In order to test the hypothesis that the high rate of dolichol synthesis in mouse testes is a result of the active division and differentiation of the various spermatogenic cell types, we measured dolichol and cholesterol synthesis in the testes of mice bearing mutations which render their testes deficient in germ cells. Mice bearing both the W (dominant spotting) mutation and the allelic mutation \(w^v\) (viable dominant spotting) to give the genotype \(W/w^v\) are viable but are severely deficient in spermatogonia, since the primary germ cells do not increase in number and do not migrate from the yolk sac to the genital ridge (14). Sertoli cell differentiation is normal in these animals (15). Mice homozygous for the mutation atrichosis \((a^t)\) are viable and also show a virtual absence of germ cells in the testes and normal Sertoli cell differentiation (15).

The average weight of testes from the various groups of mice were 14 mg/\(W/W^v\) testis versus 130 mg/++/+ testis, and

18 mg/\(a^t/a^t\) testis versus 99 mg/littermate control (+/?) testis (Table I). Nevertheless, \[^{14}C\]acetate incorporation into cholesterol by testes minces of \(W/W^v\) mice was essentially the same as for normal littermate controls. Cholesterol synthesis in \(a^t/a^t\) testes was approximately one-half of that in +/+ littermate control testes (Table I). While the extraordinarily high rate of dolichol synthesis in the testes of control mice was apparent, dolichol synthesis was diminished to barely detectable levels in the germ cell-deficient testes of the mutant mice (Table I), and the resulting ratio of dolichol synthesis to cholesterol synthesis was similar to that measured in various cell cultures (9) and in liver (Table I). In comparison with normal controls, the ratio of dolichol synthesis to cholesterol synthesis in the germ cell-deficient testes was reduced 49-fold in \(a^t/a^t\) mice and 117-fold in \(W/W^v\) mice.

Although the \(W\) and \(a^t\) mutations are located on different chromosomes \((W\) and \(W^v\) on chromosome 5; \(a^t\) on chromosome 10), we considered it possible that both of the mutations affect structural or regulatory genes concerned with dolichol

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**Table II**

Effect of \(\alpha\)-irradiation on the synthesis of dolichol and cholesterol from \[^{14}C\]acetate in mouse testes

| Time* | Weight* | Treatment | Dolichol | Cholesterol | Dolichol/cholesterol |
|-------|---------|-----------|---------|-------------|---------------------|
| mg    | dpm/testis/3 h | %       |
| 1½ days | 90 | Control | 1,437 | 81,809 | 1.76 |
|       | 89 | \(x\)-Irradiation | 1,168 | 58,792 | 1.99 |
| 28 days | 106 | Control | 1,815 | 85,788 | 2.12 |
|       | 41 | \(x\)-Irradiation | 266 | 109,093 | 0.24 |

* Two mice were killed for each determination at the indicated times following the final irradiation.

* Average wet weight per testis.

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**Fig. 2.** Light micrographs (×120 magnification) of seminiferous tubules from (a) control testes and (b) \(\alpha\)-irradiated testes 28 days after treatment. Tissue (10 to 50 mg) was fixed in Bouin's solution and sections were stained with periodic acid-Schiff's reagent and counterstained with haematoxylin. The remainder of the tissue was used to assay dolichol and cholesterol synthesis.
biosynthesis in testes. However, the rates of dolichol and cholesterol synthesis, as well as the ratio of dolichol synthesis to cholesterol synthesis in the livers of W/W and at/at mice, were essentially unchanged from those in the livers of littermate controls (Table I). Since the effects of the W and at mutations are pleiotropic, affecting blood-forming tissues (W) and the development or color of the coat (W and at), it is unlikely that their primary actions are at the level of dolichol synthesis. The ratios of dolichol synthesis to cholesterol synthesis in livers of normal and mutant mice shown in Table I are similar to those found previously in cultured cells (9) and to the levels found in testes deficient in spermatogenic cells (Table I), emphasizing the extraordinarily high rate of dolichol synthesis that appeared to be associated with spermatogenesis.

Further evidence that the low level of dolichol synthesis seen in the testes of the mutant mice was a consequence of the deficiency of spermatogenic cells was obtained by using x-irradiation to deplete mouse testes of germ cells. The abdomens of mice were shielded while their testes were exposed to x-ray doses of 300 R, followed by 100 R after a 4-day interval. Type B and mitotically active type A spermatogonia are radiation-sensitive (16) and this x-ray dose has been shown to kill spermatogonia effectively (17). At 1½ days after the final irradiation, the ratio of testicular dolichol/cholesterol synthesis was essentially unchanged from that in the control testes (Table II). Furthermore, histological examination did not indicate any obvious depletion of spermatids and spermatocytes (not shown). However, 28 days after the final x-irradiation, histological examination showed the tubules to be severely depleted of spermatids and spermatocytes (Fig. 2). The average testicular weight had decreased from 106 mg to 41 mg, and testicular dolichol synthesis was greatly reduced from the control values, while cholesterol synthesis was essentially unchanged (Table II). The ratio of [14C]fetacitetation incorporation into dolichol versus cholesterol was reduced by approximately 90% after depletion of spermatids and spermatocytes by x-irradiation (Table II).

**Discussion**

This study provides evidence for the existence of at least one spermatogenic cell type which synthesizes dolichol at a high rate by comparison with the remaining testicular cell types, other tissues, and actively growing cell cultures. Since the rate of cholesterol synthesis in germ cell-deficient testes (W/W and irradiated) is approximately equal to that in control testes or reduced by approximately half (at/at), the germ cells appear to contribute from half to none of the cholesterol synthesized in whole testes (Tables I and II). Visual inspection of the size of the seminal vesicles gave no indication that either W/W or at/at mice were testosterone deficient. However, it is possible that germ-cell-deficient testes synthesize more testosterone, and therefore more cholesterol than control testes, and cholesterol synthesis in spermatogenic cells might represent a greater fraction of the total testicular cholesterol synthesis than is apparent from the data in Tables I and II. In any case, the ratio of dolichol to cholesterol synthesis in at least one spermatogenic cell type must be higher than the ratios of 1.96 to 2.35% for whole testes. Experiments are in progress to measure dolichol synthesis in isolated spermatogenic cell types.

The high rate of dolichol synthesis in mouse testes by comparison with the rate in mouse liver mirrors the high concentration of dolichol found in human testes compared to other human tissues (12). It is of further interest that the dolichols newly synthesized by mouse testes were slightly more polar than the pig liver dolichols used as standards (Fig. 1). Since the isoprene unit of pig liver dolichol is saturated (1), it is possible that newly synthesized testicular dolichols comprise an unsaturated α unit which would render the molecules more polar (1). In this respect, it is interesting that a particular enzyme preparation from hen oviduct synthesized a polypropenyl phosphate from isopentenyl pyrophosphate and farnesyl pyrophosphate which had several properties compatible with 2,3-dehydrodolichyl phosphate (11), although it is possible that this cell-free system was incomplete for the synthesis of dolichyl phosphate possessing a saturated α-isoprene unit. If the data in Fig. 1 are indicative of an unsaturated α unit in newly synthesized testicular dolichol, this may represent an end product or it could represent an intermediate in the formation of dolichol containing a saturated α unit. Our previous studies demonstrated that mouse L cells synthesize dolichols that are chromatographically identical with pig liver dolichols (9).

This high rate of dolichol synthesis in mouse testes, which appears to be attributable to one or more spermatogenic cell types, requires further investigation. It may reflect a requirement by spermatogenic cells for a rapid rate of glycoprotein synthesis during one of the developmental stages. Glycoproteins could be necessary either for the intimate Sertoli-germ cell associations or for some later function of mature spermatozoa, such as adhesion to the zona pellucida or penetration of the oocyte. The high rate of dolichol synthesis probably does not simply reflect active cell division because such a high ratio of dolichol/cholesterol synthesis is not apparent in growing cell cultures (9).

It is of considerable interest in this regard that we have observed a sharp peak of dolichol synthesis during developing mouse brain between 3 days prenatal and 20 days after birth and that Heifetz and Lennarz (18) have reported that tunicamycin, which blocks the function of dolichyl phosphate in glycoprotein synthesis, prevents gastrulation of sea urchin embryos. In the light of these observations, it seems possible that a high rate of dolichol synthesis may be required for some as yet undefined event which occurs in the differentiation of several cell and tissue types.

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