

Acyl-CoA reductase specificity and synthesis of wax esters in mouse preputial gland tumors

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Abstract Long-chain alcohols are synthesized in the mouse preputial gland tumor (ESR-586) by NADPH:acyl-CoA oxidoreductase. In this study, a series of labeled acids was tested as substrates for the oxidoreductase in a cell-free system from the tumor, and the distribution of label into alcohols, waxes, and other products was determined. The system contained the labeled acid, an acyl-CoA-generating system, an NADPH-generating system, and tumor homogenate. The highest rates of alcohol synthesis were obtained with palmitic (16:0), heptadecanoic (17:0), stearic (18:0), myristic (14:0), elaidic (18:1 *trans*), and linoleic (18:2) acids, which yielded, respectively, 151, 124, 102, 76, 65, and 35 pmol alcohol/min per mg protein. Decanoic (10:0), lauric (12:0), oleic (18:1 *cis*), linolenic (18:3), arachidonic (20:4), and behenic (22:0) acids all gave lower activities. Acyl-CoA formation did not appear to be rate limiting with any of the substrates tested except behenic acid. In addition to the fatty alcohol product, a small amount of fatty aldehyde was formed in the system. Incorporation of the labeled fatty acids into wax esters was examined and the distribution of label between the alcohol and acid components of the waxes was determined. Incubation of [1-¹⁴C]palmitic acid yielded 3.4% free alcohol, 8.3% alcohol esterified in waxes, and 7.7% palmitoyl groups esterified into waxes, whereas, at the other extreme, [1-¹⁴C]linolenic acid yielded 0.8%, 0.6%, and 38%, respectively, into the homologous components.—**Wykle, R. L., B. Malone, and F. Snyder.** Acyl-CoA reductase specificity and synthesis of wax esters in mouse preputial gland tumors. *J. Lipid Res.* 1979. **20:** 890–896.

Supplementary key words NADPH:acyl-CoA oxidoreductase · fatty alcohol biosynthesis · wax synthesis · alkyl lipid precursors

The synthesis of fatty alcohols (1, 2), wax esters (2, 3), and the alkyl ether-linked glycerolipids (4–8), which are characteristically elevated in tumors (9), has been examined in a number of studies of the mouse preputial gland tumor (ESR-586). The tumor appears to be unique in that fatty alcohols and wax esters are barely detectable in the tumors until 3 weeks after they are transplanted, but then the wax esters increase markedly to become the major lipid class (10); at the same time, the ether lipids and free alcohols also increase (10, 11). Similar increases have been observed when cloned cells from the tumor reach confluency

(12). The preputial gland tumor thus provides a useful system for studying factors that control the synthesis of fatty alcohols, waxes, and ether lipids.

In mammalian tissues, the long-chain alcohols are precursors of waxes (2–4, 13) and of both the alkyl- and alk-1-enyl-linked glycerolipids (14). The alcohols in turn are synthesized from the corresponding fatty acids (14) as they are in birds (15), marine organisms (16), plants (17), and bacteria (18). Previous studies indicated that the synthesis of fatty alcohols is catalyzed by an NADPH:acyl-CoA oxidoreductase in the preputial gland tumor (1). In most tissues, the composition of the ether chains is simple and consists almost entirely of 16:0, 18:0, and 18:1 groups (19), but in the preputial gland tumor, there is a wider range of chain lengths with up to half the alkyl chains being longer than 18 carbons in older tumors (10). Fewer long-chain ethers were found in younger (3 weeks after transplant) tumors (5). The alcohol composition of the waxes is somewhat different and consists mostly of alcohols having 18 or fewer carbon atoms (10).

The enzyme that catalyzes formation of the ether bond from acyldihydroxyacetone phosphate and fatty alcohol utilizes a wide range of alcohols and does not appear to possess the specificity to account for the narrow spectrum of chains normally found in the ether lipids (8, 14, 20–26). The composition of the ether lipids, therefore, appears to be determined primarily at the point of fatty alcohol formation; this conclusion is supported by recent studies using microsomes from rat brain (24–26). However, control of the composition of the ether lipids in the preputial gland tumor appears to be more complex, since the alcohol is incorporated into both wax esters and ether-linked lipids. The present study was undertaken to examine the specificity of the NADPH:acyl-CoA oxidoreductase in the preputial gland tumor and to determine if the

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wide range of alcohol and ether chains found in the tumor lipids can be attributed to a broader than normal specificity of the reductase.

MATERIALS AND METHODS

The original tumors were obtained from The Jackson Laboratory, Bar Harbor, ME, and were grown in C57BL/6 mice as described earlier (4). Co-A, ATP, and NADP were purchased from P-L Biochemicals, Milwaukee, WI; glucose-6-phosphate and glucose-6-phosphate dehydrogenase were from Sigma Chemical Co., St. Louis, MO.

The following labeled fatty acids were obtained from New England Nuclear Corp.: [^{14}C]lauric (12:0), 9.5 mCi/mmol; [^{14}C]myristic (14:0), 4.3 mCi/mmol; [^{14}C]palmitic (16:0), 55 mCi/mmol; [^{14}C]stearic (18:0), 55 mCi/mmol; [^{14}C]oleic (18:1 *cis*), 55 mCi/mmol; and [5,6,8,9,11,12,14,15- ^3H](*N*)arachidonic (20:4), 80 Ci/mmol. The others were obtained from Applied Science: [^{14}C]decanoic (10:0), 58 mCi/mmol; [^{14}C]heptadecanoic (17:0), 55 mCi/mmol; [^{14}C]linoleic (18:2), 40–60 mCi/mmol; [^{14}C]linolenic (18:3), 53 mCi/mmol; and [U- ^{14}C]behenic (22:0), 500–630 mCi/mmol. [^{14}C]Palmitoyl-CoA (61 mCi/mmol) was obtained from New England Nuclear Corp. The labeled acids were all greater than 98% pure as determined by thin-layer chromatography. The corresponding unlabeled fatty acids were obtained from: Analabs, Inc., New Haven, CT (12:0, 14:0, 17:0, 18:0, 22:0, 18:1 *cis*, and 18:1 *trans*); Applied Science Laboratories, State College, PA (18:2, and 18:3); and Nu Chek Prep., Inc., Elysian, MN (20:4 and 8,11,14-eicosatrienoic acid, 20:3). Some of these were mixed with the labeled acids in chloroform to give substrates of lower specific activities for use in incubations.

Preparation of subcellular fractions

Tumors (approximately 7 g each) were harvested 8 weeks after transplant; homogenation was carried out as described earlier (4). The assays were carried out using the 500 *g* × 10 min supernatant, which was stored at -27°C until immediately before addition to assay mixtures. Protein was determined by the procedure of Lowry et al. (27).

Enzyme assays and extraction of products

The standard reaction mixture contained the following components to effect acyl-CoA formation: the labeled fatty acids (40 μM added in 20 μl of ethanol), ATP (10 mM), CoA (0.1 mM), and MgCl_2 (4 mM). In addition the reaction mixture contained 0.1 M Tris-

HCl buffer (pH 7.1) and an NADPH-generating system consisting of NADP $^+$ (2 mM), glucose-6-phosphate (6 mM), and glucose-6-phosphate dehydrogenase (2.5 units). Each fatty acid was assayed at three different concentrations of the tumor homogenate: 0.83, 1.7, and 3.3 mg protein/ml. The mixtures were shaken in a Dubnoff metabolic shaker at 37°C for 10 min in a final volume of 2 ml. When all labeled products were to be measured, incubations were stopped by extracting the lipids using the procedure of Bligh and Dyer (28), modified by including 2% acetic acid in the methanol. Labeled products in the upper aqueous phase were measured and contained the acyl-CoA synthesized. Alternatively, when only the total amount of alcohol synthesized was determined, the incubations (2 ml) were stopped by adding 4.8 ml of 1.8 N KOH in ethanol to the incubation mixture; the tubes were sealed and heated for 2 hr in a boiling water bath. Additional water (5.7 ml) was added and the mixture was acidified with 1.5 ml of 6 N HCl. The lipids were then extracted three times into 5-ml portions of chloroform. Under these conditions, 97% of the label added to the saponification mixture was recovered; similar recoveries were observed when purified wax esters were saponified. After the total incubation mixtures were saponified, most of the label was found in fatty acids and fatty alcohols, e.g., when [^{14}C]palmitate was incubated with 3.3 mg protein/ml under standard conditions, 12% of the label was in fatty alcohol, 80% was in fatty acid and hydroxy fatty acid, 1% was in alkylglycerol, and 6% was in a product migrating with methyl palmitate; a small amount of label was in unidentified products possibly derived from the aldehyde. In this procedure, >98% of pure wax esters were hydrolyzed; saponification of the wax esters yielded only fatty alcohols and fatty acids.

Determination of products

Labeled products were separated by thin-layer chromatography and the distribution of label was determined by zonal profile scanning and area scraping procedures (29, 30). Total lipids were separated on layers of Silica Gel G developed in hexane–diethyl ether–glacial acetic acid 70:30:1 (v/v). When total alcohol synthesis was measured by saponification as described above, the saponification products (fatty alcohols, fatty acids, and hydroxy fatty acids) were developed in chloroform–methanol–ammonium hydroxide 98:2:1 (v/v). In this system, fatty acids and hydroxy fatty acids remain at the origin below the fatty alcohols. The saponification products were also developed in hexane–diethyl ether–acetic acid 60:40:1 (v/v) to identify the fatty acid. Purified wax esters were

TABLE 1. Major products after incubating labeled fatty acids with homogenates of mouse preputial gland tumors^a

| Labeled Acids Incubated | Percent of Added Label | | | | | |
|-------------------------|------------------------|-------------------|--------------|-----------|--------------|-----------------------------|
| | Acyl-CoA | Unesterified Acid | Free Alcohol | Wax Ester | Phospholipid | Other Products ^b |
| 10:0 | 13 | 83 | 0.6 | 0.6 | 0.6 | 2.2 |
| 12:0 | 19 | 71 | 0.6 | 4.2 | 4.3 | 0.9 |
| 14:0 | 17 | 27 | 1.9 | 21 | 16 | 17 |
| 16:0 | 20 | 15 | 3.4 | 16 | 26 | 19 |
| 17:0 | 28 | 28 | 6.3 | 15 | 17 | 6 |
| 18:0 | 22 | 46 | 5.1 | 5.4 | 13 | 9 |
| 22:0 | 0 | 99 | 0 | 0.3 | 0 | 0 |
| 18:1 <i>trans</i> | 30 | 37 | 3.5 | 9.5 | 12 | 8 |
| 18:1 <i>cis</i> | 27 | 46 | 1.5 | 7.9 | 12 | 6 |
| 18:2 | 24 | 26 | 1.0 | 21 | 12 | 16 |
| 18:3 | 14 | 27 | 0.8 | 39 | 6 | 13 |
| 20:4 | 13 | 55 | 0.7 | 3.0 | 13 | 15 |

^a Fatty acids (80 nmol) were incubated in standard incubations containing 3.3 mg protein per ml; products were extracted and identified as described in Methods. Values are averages from duplicate incubations.

^b Other products included triacylglycerols, a product that chromatographed with aldehydes and methyl (or ethyl) esters of fatty acid, and another product tentatively identified as hydroxy fatty acid.

obtained by separating them from other products on layers of Silica Gel G developed in benzene and eluting them from the appropriate band with chloroform. The purified wax esters were saponified by the procedure outlined above and the alcohols and acids released were separated on layers of Silica Gel G developed in hexane–diethyl ether–acetic acid 60:40:1 (v/v). Identification of acyl-CoA by thin-layer chromatography was based on its migration with authentic palmitoyl-CoA on layers of Silica Gel G developed in either methanol or in butanol–acetic acid–water 5:2:3 (v/v). Alcohols were identified by thin-layer chromatography and by gas–liquid chromatography of the acetate derivatives. Acetates of isolated fatty alcohols were prepared by heating the fatty alcohols for 1 hr at 100°C in a sealed tube with 2 ml of acetic anhydride–pyridine 4:1 (v/v). The fatty alcohol acetates were separated by gas–liquid chromatography (31) and the radioactive materials were collected from the peaks as described earlier (32). The dimethylacetal derivatives of the isolated fatty aldehydes were prepared by acid methanolysis (33, 34) and analyzed by gas–liquid chromatography under the same conditions described previously for the analysis of methyl esters of fatty acids (32).

Endogenous fatty acids were measured by extracting the enzyme preparation by the procedure of Bligh and Dyer (28) modified by the presence of 2% acetic acid in the methanol. Free fatty acids were isolated from total lipids by thin-layer chromatography, methyl esters were prepared, and the distribution of mass was determined by gas–liquid chromatography (32).

RESULTS

The levels of free acid in the enzyme preparation were determined before and after incubation. The acids occurring at the highest level (in an incubation mixture containing 3.3 mg protein/ml) before incubation were 18:1 (35 nmol), 16:0 (19 nmol), 20:4 (13 nmol), 18:2 (12 nmol), and 18:0 (8 nmol). To test for the release of fatty acids during incubations, the preparation was incubated for 10 min at 37°C in 0.1 M Tris buffer (pH 7.1) containing MgCl₂ (5 mM) and no other cofactors; ATP and CoA were omitted to minimize reutilization of released acid. A total of 36 nmol of fatty acids having the same distribution as in the unincubated preparation was released during the incubation.

Optimal conditions for the synthesis of alcohol were established using [1-¹⁴C]palmitate as the substrate. The pH optimum was 7.1 and the optimal substrate concentration was 40 μM. Under the optimal conditions, the rate was linear from 5 to 15 min. In 5-min incubations the rates were linear with protein concentrations up to 3.3 mg/ml for each of the substrates compared. Since the substrates were added in 20 μl of 95% ethanol, 10–50 μl of ethanol per incubation were tested and found to have no detectable effect on fatty alcohol formation. No synthesis of fatty alcohol from [1-¹⁴C]palmitate was observed in the absence of the acyl-CoA-generating system. The NADPH-generating system used was more effective than NADPH alone for the synthesis of alcohols. When NADPH (1.7 mM) was substituted for the NADPH-generating

system in the standard incubation mixture, the conversion of [^{14}C]palmitic acid to alcohol was reduced to 28% that of the control containing the generating system; at 7 mM NADPH the synthesis of alcohol was 72% that of the control. In the absence of NADPH, the synthesis of alcohol from the most active substrates (16:0, 17:0, and 18:0) was <1 pmol/min per mg protein. [^{14}C]Palmitoyl-CoA (4 μM) was tested as a substrate in the absence of added ATP, CoA, and Mg^{2+} but only 2% of the label, or 3 pmol/min per mg protein, was incorporated into fatty alcohol. The enzyme preparation contained an active acyl-CoA hydrolase that hydrolyzed >95% of the [^{14}C]palmitoyl-CoA (4 μM) within 10 min; at 150 μM [^{14}C]palmitoyl-CoA, 50% was hydrolyzed in 10 min. In the presence of the acyl-CoA-generating system, acyl-CoA accumulated (Table 1); thus the rate of synthesis of acyl-CoA appears to be considerably higher than the rate of acyl-CoA hydrolysis during the 10-min incubation; the acyl-CoA synthesized endogenously may also be less accessible to the hydrolase.

After extraction of the lipids, most of the acyl-CoA formed (>95%) remained in the upper aqueous phase; this was demonstrated by adding authentic [^{14}C]palmitoyl-CoA to the incubation mixture and extracting. When [^{14}C]palmitic acid was used as the substrate, the labeled product in the upper phase was identified as acyl-CoA by several criteria. First, no

activity was found in the upper phase unless ATP and CoA were present in the incubations. Second, saponification of the product yielded (after acidifying) the free acid. Finally, the product chromatographed with authentic acyl-CoA on silica gel layers (see Methods). The amounts of labeled acyl-CoA found at the end of 10-min incubations of the series of acids tested are shown in Table 1. Acyl-CoA was formed from all the fatty acids except 22:0.

Several glycerolipids, mainly phospholipids and triacylglycerols, were formed in the incubations and are listed in Table 1. Free fatty alcohol was present, but much of the alcohol synthesized was incorporated into wax esters. The distribution of label among products varied considerably from substrate to substrate, but with all the acids tested, except 22:0, free acid and acyl-CoA remained at the end of the incubation.

With most of the substrates tested, a small amount of label (4% for the 16:0 acid) was found in the fraction containing fatty aldehydes and methyl esters of fatty acids. This fraction was isolated from incubations containing the 16:0 substrate, and after acid methanolysis (33, 34), 12% of the activity in the fraction was found as dimethylacetals of the fatty aldehydes and 88% as methyl esters of the fatty acids. The dimethylacetal fraction was isolated and analyzed by gas-liquid chromatography (32); approximately 80% of the injected radioactivity was recovered with 90% in the fraction

TABLE 2. Incorporation of labeled fatty acids into wax esters and acyl-CoA reductase activities with different substrates

| Labeled Acids Incubated | Percent of Labeled Substrates Incorporated into Wax Esters ^a | Distribution of Label in Alcohol and Acid Components of Wax Esters ^b | | Acyl-CoA Reductase ^c pmol of Alcohol Formed/min per mg protein |
|-------------------------|---|---|-----------------|---|
| | | Percent in Alcohol | Percent in Acid | |
| 10:0 | 0.6 | 6.5 | 93 | 1 |
| 12:0 | 4.2 | 5.5 | 94 | 3.6 |
| 14:0 | 21 | 15 | 85 | 76 |
| 16:0 | 16 | 52 | 48 | 151 |
| 17:0 | 15 | 23 | 77 | 124 |
| 18:0 | 5.4 | 31 | 69 | 102 |
| 22:0 | 0.3 | | | <1 |
| 18:1 <i>trans</i> | 9.5 | 26 | 74 | 65 |
| 18:1 <i>cis</i> | 7.9 | 4.7 | 95 | 8.5 |
| 18:2 | 21 | 6.8 | 93 | 35 |
| 18:3 | 39 | 1.6 | 98 | 14 |
| 20:4 | 3.0 | 8.2 | 92 | 14 |

^a Percentages are based on the amount of the fatty acids (80 nmol) added to the incubation mixtures and are for standard incubations containing 3.3 mg protein per ml.

^b Wax esters were isolated from incubations containing 1.7 mg protein per ml. The purified wax esters were saponified, the products were extracted from the acidified mixture, and the radioactivity was measured in the released acid and alcohol after separation by thin-layer chromatography. The recovery of label from the saponification mixture was >97%; >98% of the recovered label was in fatty acids and fatty alcohols, distributed as shown.

^c Incubations were carried out and alcohol formation was measured (after saponification) as described in Methods. Values are from the slopes of plots of activities at three protein concentrations (0.83, 1.7, and 3.3 mg per ml) incubated (in duplicate for each protein concentration) as described for standard incubations in Methods.

corresponding to the 16:0 aldehyde. Based on these results, the incubation products contained approximately 0.5% [¹⁴C]palmitaldehyde.

The labeled wax fraction formed from each of the fatty acids was purified and hydrolyzed to determine the distribution of label between the acid and alcohol components of the esters (Table 2). With the 16:0 substrate, about half the label in the wax fraction was present in the alcohol and about half in the acid, but with others, such as 18:3, almost all of the label was in the acid portion of the molecule. The 18:3 acid was most actively incorporated into the wax esters, but 18:2, 14:0, 16:0, and 17:0 were also good substrates. The distribution of label between the alcohol and acid portions of the wax for the 18:1 *trans* acid resembled that obtained with the 18:0 acid rather than with the 18:1 *cis*.

Fatty alcohol synthesis from the series of fatty acids is shown in Table 2. Highest activities were obtained with the 16:0, 17:0, and 18:0 and the lowest activities with the 10:0, 22:0, and 12:0 acids. Only traces of labeled ether lipids were detected in the products and none would be expected, since the presence of NADPH inhibits the synthesis of ether lipids from fatty alcohols (14). Radiopurities (based on gas-liquid chromatography) of the alcohol acetates derived from the following respective substrates were: 14:0, 98%; 16:0, 95%; 17:0, 97%; 18:0, 97%; 18:1 *trans*, 83%; and 18:2, 96%. These data demonstrate that the alcohols formed have a structure analogous to that of the parent acid and little, if any, chain elongation or desaturation occurred.

DISCUSSION

Alcohol formation was highest with the following substrates, listed in decreasing order of their rates of conversion: 16:0 > 17:0 > 18:0 > 14:0 > 18:1 *trans* > 18:2. Concentrations of these alcohols found in the wax esters of older tumors were 16:0 > 18:0 > 14:0 > 17:0; the 16:0 alcohol comprised 35% and 17:0 comprised 4.4% of the wax-bound alcohols; there is also a much higher level of the 16:0 than the 17:0 chains in the ether lipids (10). The cellular concentration of the 17:0 alcohol is, therefore, much lower than would be expected from its high rate of enzymatic formation. Thus, the availability of the 17:0 acid substrate probably limits the level of this alcohol in the tumor. No 18:1 *trans* alcohol normally is found in tissues and little, if any, 18:2 alcohol is present in the tumor lipids (5, 10). For the other substrates, the rates of conversion paralleled the relative concentrations of the respective alcohols found in the tumors.

Since 80 nmol of the labeled substrates was added in standard incubations, the maximum dilution possible of the individual acids by endogenous fatty acids was less than 20% at the lowest protein concentration (0.83 mg/ml). At the highest protein concentration (3.3 mg/ml), the maximum dilutions possible (including the fatty acid released during the incubations) were as follows: 16:0, 34%; 18:0, 14%; 18:1 *cis*, 62%; 18:2, 21%; and 20:4, 23%. Mixing of various unlabeled acids (40 nmol) with [1-¹⁴C]palmitic acid (40 nmol) had little effect on the rate of conversion of the labeled acid to the alcohol (data not shown). No effect of dilution of the substrates was observed when different levels of the enzyme preparation were used, i.e., incorporation of label into alcohol was linear for each acid over the range of enzyme concentrations used. Dilution of substrates, therefore, does not appear to be a serious problem in the system. It is not clear to what degree the exogenous substrates are mixed with the endogenous fatty acids during the incubation period.

In the alkyl-ether chains of the lipids of older tumors, the 22:1 + 22:0 fraction was the most abundant of all the species (10). In the present study the 22:1 acid was not tested as a substrate but the 22:0 was, and it was not converted to the alcohol. This nonconversion is explained by the lack of acyl-CoA formation with the 22:0 substrate (Table 1). Differences for the longer chain substrates may persist *in vivo*. It was suggested by Grigor (10) that the alkyl ether chains and wax chains may be derived from different alcohol pools, since the alkyl lipids appear in transplanted tumors before the wax esters and have longer chains than those of the waxes. A coupling of fatty alcohol synthesis to fatty acid synthesis has been described in *Euglena* (35) and in the rabbit harderian gland (36). In these systems, newly formed fatty acids released from the fatty acid synthase are reduced much more readily than exogenous substrates. It is possible that a system similar to this is also involved in the synthesis of the longer chain alcohols in the preputial gland tumor.

Earlier studies of the lipids of the normal mouse preputial gland revealed a close structural relationship between the free alcohols and the ether-linked lipids (31). The composition of the alcohol and ether chains of the preputial gland is simpler than that of the tumor. Polyunsaturated groups represent a negligible proportion in each case. However, the gland contains only 8–10 different chains (mainly C₁₄ and C₁₆) (31), whereas the older tumors contain at least 23 different chains (C₁₄ to C₂₄) (10). This indicates that the enzyme system that synthesizes the alcohols in the tumor has lost much of its specificity. Whether this results from the presence of more than one acyl-CoA reductase in these tissues or if a single reductase is present whose

specificity is regulated by factors such as membrane fluidity is not known and requires further study.

The simple pattern of chains found in the ether-linked lipids of most tissues likely results from more highly specific alcohol-synthesizing enzymes in those tissues (24–26, 36, 37). The partially purified NADH-dependent acyl-CoA reductase isolated from the soluble fraction of bovine cardiac muscle by Johnson and Gilbertson (37) utilized both 16:0 and 18:0 acyl-CoAs but not the 14:0 or 18:1 substrates. The ether-linked chains found in bovine heart consist predominantly of the 16:0 species but 18:0 and 18:1 *cis* species are also present (19, 38). In a recent study using rat brain microsomes, the 18:1 *cis* acid was found to be a slightly better substrate for alcohol synthesis than the 16:0 acid (26), but in the present study, the 18:1 *cis* acid was converted at only 5.6% the rate of the 16:0 acid. These differences in specificity in the brain and tumor correlate well with the distribution of chains found in the tissues. In rat brain the 18:1 species comprises approximately 40% of the plasmalogens (19), which are derived from the alcohols, whereas in the mouse preputial gland tumor only 1.3% of the alcohols of the wax esters and 3.4% of the alkyl chains are of the 18:1 species (10). As Bishop and Hajra (24) found with rat brain microsomes, acyl-CoA formation did not appear to be rate limiting in the tumor system for any of the substrates tested except 22:0. Assuming that incorporation into all products occurred via acyl-CoA formation, then in most cases over half the substrate was activated in the course of the incubations (Table 1). The acyl-CoA reductase activity is higher in the preputial gland tumor (151 pmol/min per mg protein for 16:0, Table 2) than in the brain where the activity was 5–8 pmol/min per mg protein for the 16:0 acid (24, 26).

The specific pattern of chains found in the alcohols, wax esters, and ether-linked lipids of the preputial gland tumor can be attributed to the relatively broad specificity of the alcohol-synthesizing enzyme system in the tumor and the availability of fatty acids. However, even though the specificity was broad, there was still considerable discrimination among the substrates. Despite this apparent explanation of the observed composition of the alkyl ethers and wax esters in the tumor, alternate systems can be invoked to account for the different compositions observed in the normal gland and tumor, e.g., if alcohol synthesis can be coupled directly to fatty acid synthesis in the tumor (not yet demonstrated), a change in *de novo* synthesis of fatty acids or chain-elongation systems to produce longer fatty acyl chains coupled to reduction could account for compositional changes. The factors that initiate alcohol synthesis 3 weeks after the tumors are transplanted and those that determine whether alcohols

go into waxes or ether-linked lipids require further study.□□

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