TUMOR LIPIDS: BIOSYNTHESIS OF PLASMALOGENS[1]

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SUMMARY

Labeled 1-^{14}C-1-^{3}H-hexadecanol was incubated *in vivo* with Ehrlich ascites cells for 24 and 48 hours. The major distribution of radioactivity was found in the acyl, alkyl, and alk-1-enyl moieties of phosphatidyl choline, phosphatidyl ethanolamine, and glyceryl ether diesters. The alkyl moiety of each class contained the highest percentage of activity that decreased at 48 hours. A concomitant increase in the activity of the alk-1-enyl moiety paralleled the decrease in the activity of the alkyl glyceryl ether. The $^{3}H/^{14}C$ ratio of the alkyl fraction was similar to that of the administered alcohol, but the $^{3}H/^{14}C$ ratio of the alk-1-enyl ether moieties was reduced approximately one half. The data indicate that plasmalogens are derived from the corresponding alkyl acyl phosphatides by biodehydrogenation.

Glyceryl ether diesters of neoplasms were first identified in lipids of Ehrlich ascites cells (1). More recently we demonstrated that phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE) of this neoplasm contain a high percentage of alkyl and alk-1-enyl glyceryl ether moieties (2). The neutral lipids and phospholipids of several transplantable rat and mouse tumors (3) and a number of human neoplasms (4) have also been shown to contain a relatively high level of alkyl and alk-1-enyl glyceryl ethers. The significance of these observations remains obscure because little is known of the origin and function of the ether-linked lipids.

Friedberg and Greene (5) and later Ellingboe and Karnovsky (6), using marine organisms in *in vitro* experiments, obtained evidence to suggest that

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long-chain alcohols could serve as precursors of alkyl glyceryl ethers. Recently Snyder *et al.* (7,8) and Kapoulas and Thompson (9), using a cell-free system, confirmed this finding. Despite the *in vitro* experiments, long-chain alcohols have not been shown to be precursors of alkyl glyceryl ethers in *in vivo* experiments although attempts have been made (3,10,11). Numerous investigators, Hagen and Goldfine (12), Ellingboe and Karnovsky (6), Carr *et al.* (13), Bickerstaffe and Mead (14), Bell and White (15) and others have attempted to demonstrate that aldehydes are precursors of alk-1-enyl glyceryl ethers, but the results have been inconclusive and often conflicting. Until now, the route of alk-1-enyl glyceryl ether biosynthesis was best described as unknown. The data presented in this report suggest the origin of the alk-1-enyl acyl PC and PE *(plasmalogens)*.

EXPERIMENTAL

Ehrlich ascites cells were grown in the peritoneal cavity of white Swiss mice (5 or 6 animals)(HA/ICR strain). The dose [approximately 2 to 2-1/2 μc (^{14}C) of 1-^{14}C-1-^{3}H-hexadecanol, specific activity (^{14}C) 39 and (^{3}H) 334 μc/mg] was dispersed in an aqueous solution of Tween-20 (2%) and administered (1/2 ml) by interperitoneal injection on the sixth day after transplantation. Cells and fluid were harvested 24 and 48 hours after administration of the dose, and extracted (16); lipid classes were isolated by thin-layer chromatography (TLC) (2) and reduced with $LiAlH_4$ (17). The distribution of activity in alkyl, alk-1-enyl, and alcohol moieties was determined by counting successive 2-mm sections removed from developed chromatoplates (18). Gas-liquid chromatography (GLC) and TLC conditions have been described (2,17). Labeled 1-^{14}C-1-^{3}H-hexadecanol was prepared by reduction of 1-^{14}C-hexadecanoic acid with $LiAl^{3}H_4$.

RESULTS AND DISCUSSION

Approximately 30% of the administered labeled alcohol was found in the lipids of the Ehrlich ascites cells and fluid after incubation at both time periods. Approximately 90% of the activity was found in the GEDE, PC , and

TABLE I

Distribution of Activity Between Acyl, Alk-1-enyl, and Alkyl Moieties after *in vivo* Incubation of 1-^{14}C-^{3}H-Hexadecanol with Ehrlich Ascites Cells for 24 and 48 hours.†

Incubation Time	Lipid Class	% Distribution of Activity (^{14}C) into		
		Acyl‡	Alkyl	Alk-1-enyl
24 hours	GEDE	9	83	1
	PC	27	67	2
	PE	13	73	11
48 hours	GEDE	12	76	4
	PC	32	57	11
	PE	20	48	32

†Distribution of activity determined by counting successive 2 mm sections of adsorbent removed from a developed chromatoplate after the individual lipid classes had been reduced with $LiAlH_4$. The lack of the sums of the activities in these areas to equal 100 is due to minor amounts of activity in other areas of the chromatoplate.

‡Acyl percentages are represented by the activity in the long-chain alcohol area.

PE fractions. None of the administered free alcohol could be detected in the extracted lipids after 24 hours. The percentage distribution of activity in acyl, alkyl, and alk-1-enyl moieties of each class containing ether-linked lipids (GEDE, PC, and PE) after 24- and 48-hour incubations are given in Table I. The alkyl moiety of all three lipid classes contained the highest percentage of activity at both times. At 24 hours, the alk-1-enyl glyceryl ethers contained little activity but after 48 hours they contained a high percentage, especially PE, which contains a high percentage of plasmalogens (2). The increase in the percentage of activity found in the alk-1-enyl moiety with increase in time is illustrated in Figure 1. A concomitant decrease in the percentage of activity found in the alkyl moiety paralleled the increased activity of the alk-1-enyl fraction at 48 hours (Table I). These were our first results indicating

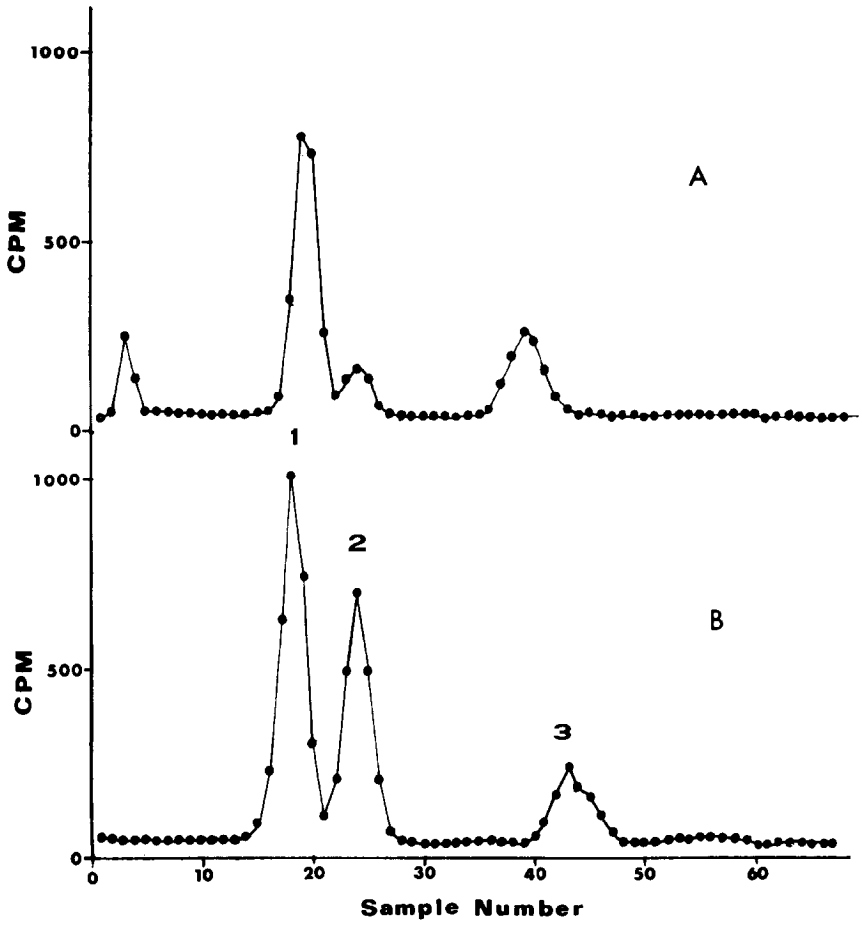

Figure 1. Distribution of carbon-14 activity in alkyl (Peak 1), alk-1-enyl (Peak 2), and alcohol (originally acyl)(Peak 3), moieties of PE after 24 (A) and 48 hour (B) *in vivo* incubation of labeled alcohol with Ehrlich ascites cells. Distribution determined as described in Table I.

that alkyl glyceryl ethers give rise to the alk-1-enyl ethers. The percentage of activity in the acyl fraction did not change significantly from the 24- to 48-hour period, indicating that the dehydrogenation of the alkyl moiety occurred on the intact molecule. A faster turnover rate of the acyl and alkyl fractions than those of the alk-1-enyl moiety could give rise to an apparent increase in the percentage of activity in the alk-1-enyl moiety as observed. However, this possibility is negated by the fact that the specific activity of the alkyl moiety of PC and PE decreased on the average only

approximately 35% from the 24 to the 48 hours, while a 3-5 fold percentage increase in the activity of the alk-1-enyl ether fraction was observed for the same period.

The $^{3}H/^{14}C$ ratios found in the acyl, alkyl, and alk-1-enyl fractions derived from each lipid class are shown in Table II for both periods. The ratios did not change significantly in any fraction of any lipid class with change in time. Tritium/Carbon-14 ratios of the alkyl moiety for each class were approximately the same as that of the administered alcohol. These data demonstrate the incorporation of long-chain alcohols into alkyl glyceryl ether without oxidation. The $^{3}H/^{14}C$ ratio of the alk-1-enyl ether fraction was reduced approximately one half that of the administered alcohol. The 50% loss of tritium in the alk-1-enyl ethers and the decrease in the activity of the alkyl glyceryl ethers offset by a corresponding increase in the activity of the alk-1-enyl ether fraction after 48 hours led us to the following conclusion: biodehydrogenation of alkyl acyl PC and PE results in the formation of plasmalogens of the respective lipid classes. Oxidation of the alcohol to an aldehyde, followed by incorporation into alk-1-enyl glyceryl ethers, would also give a $^{3}H/^{14}C$ ratio reduced by one half. Preliminary data obtained with a double-labeled aldehyde show its disappearance within 24 hours, and also a 50% loss of tritium from the alk-1-enyl moiety suggesting that the decreased $^{3}H/^{14}C$ ratio in the alk-1-enyl moiety is not due to oxidation of the alcohol to the aldehyde. Fractionation of the alkyl and alk-1-enyl hydrocarbon chain according to chain length and degree of unsaturation by GLC showed that 85 to 95% of the activity was in the 16:0 fraction. This minimizes the possibility of complete degradation and resynthesis of alkyl and alk-1-enyl glyceryl ethers containing the $^{3}H/^{14}C$ ratios observed.

These data demonstrate the *in vivo* incorporation of long-chain alcohols preferentially into alkyl glyceryl ethers of GEDE, PC, and PE and agree with the *in vitro* work of Friedberg and Greene (5), Ellingboe and Karnovsky (6), Snyder *et al.* (7,8), and Thompson (9). Previous failure to recognize significant

TABLE II

Comparison of ^{3}H to ^{14}C Ratios in Acyl and Ether-Linked Moieties of Various Lipid Classes after *in vivo* Incubation of 1-^{14}C-1-^{3}H-Hexadecanol with Ehrlich Ascites Cells†

Incubation Time	Lipid Class	$^{3}H/^{14}C$ Ratio‡		
		Acyl	Alkyl	Alk-1-enyl
24 hours	GEDE	1.5	7.1	---
	PC	0.6	7.3	4.4
	PE	1.0	7.4	4.4
48 hours	GEDE	2.0	8.0	3.6
	PC	0.4	8.2	3.2
	PE	1.6	9.1	3.6

†Distribution of activity determined as described in Table I.

‡Tritium/carbon-14 ratio of the administered alcohol was 8.57.

incorporation of long-chain alcohols into the alkyl moieties of Ehrlich ascites cells is not clear (3). On the basis of detailed structural analyses of Ehrlich ascites cell triglycerides, GEDE, PC, and PE (2), we previously were able to show a relation between the two types of ether-linked moieties and suggestive evidence to indicate that the alkyl ethers gave rise to the alk-1-enyl glyceryl ethers. Similar suggestive evidence to indicate that alkyl glyceryl ethers give rise to the corresponding alk-1-enyl ethers has been obtained by Thompson (19), Horrock and Ansell (20), and Malins (21); but like our earlier data (2), these results were inconclusive. The present data would appear to demonstrate that alk-1-enyl acyl PC and PE *(plasmalogens)* are derived from the corresponding alkyl acyl phosphatides by biodehydrogenation. The alk-1-enyl diacyl glycerides *(neutral plasmalogens)* could be derived from GEDE in an analogous manner.

Although our data appear to support only the proposed biosynthetic pathway, other pathways have been proposed on the basis of data obtained in

other tissues and organisms that may possess an alternate biosynthetic route. Both *in vivo* and *in vitro* experiments using double-labeled hexadecanal and labeled hexadeconoyl-CoA in addition to other alcohol experiments are now being carried out with this neoplasm.

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