ALTERATION OF THE FATTY ACID COMPOSITION OF EHRLICH ASCITES TUMOR CELL LIPIDS[1]

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Received February 20,1975

SUMMARY: The fatty acid composition of Ehrlich ascites tumor lipids was altered markedly in vivo by changing the type of fat fed to the tumor-bearing mice. As compared with regular chow, large differences were produced in polar and neutral lipid fatty acyl groups when the tumor cells were grown in mice fed coconut oil, sunflower oil or fat deficient diets. Subcellular membrane fractions obtained from these cells exhibited similar variations in fatty acyl composition. This experimental system provides large quantities of malignant cells for study of the relationships between membrane lipid structure and function.

A widely used procedure for investigating structure-function relationships in biological membranes is to determine the effects of changes in fatty acid composition on membrane properties. Most of the initial studies were done with mycoplasma (1,2) and bacteria (3-5). Recently, this work has been extended to mammalian cells in long-term culture (6-8). Three lines of evidence suggested that it might be possible to produce similar changes in ascites tumor cells during tumor growth in vivo. Early work indicated that dietary lipids are incorporated into rodent tumors (9) and that the type of dietary fat influenced the degree of unsaturation of the tumor lipids (10-13). Furthermore, the fatty acid composition of hepatoma cells in long-term culture changes when the type of lipid in the culture medium is varied (14, 15). Finally, studies with Ehrlich ascites tumor cells indicated that extracellular fatty acids are rapidly taken up (16-18) and that there is a rapid turnover of intracellular phospholipid fatty acyl groups (19). The present work demonstrates that large alterations in the fatty acid composition of the Ehrlich

[1]Supported by research grant HL-14781 from the National Heart and Lung Institute.

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Table I Effects of dietary fat on the fatty acid composition of Ehrlich ascites tumor cell lipids

Mice were placed on the diet 50 days prior to inoculation of the tumor, and the cells were obtained 12 days after transplantation.

	Percentage Composition							
	Polar Lipids				Neutral Lipids			
Fatty Acid[a]	Regular	Coconut Oil	Sunflower Oil	Fat Deficient	Regular	Coconut Oil	Sunflower Oil	Fat Deficient
				%				
14:0	nd[b]	2.6	nd	2.6	1.3	4.0	1.0	2.6
16:0	13.8	16.5	16.6	13.8	14.3	15.6	11.5	15.8
18:0	19.2	14.5	24.5	13.6	10.3	7.9	8.3	8.1
16:1ω7	1.5	4.9	tr[c]	5.1	1.6	4.5	1.0	4.6
18:1ω9	19.1	35.4	12.1	47.0	24.7	44.2	20.2	61.7
20:1ω9	1.2	2.0	0.9	2.2	2.1	5.4	2.5	6.7
18:2ω6	19.1	9.3	24.4	1.5	20.6	6.0	34.0	1.8
20:3ω9	1.4	2.8	1.0	8.8	tr	1.5	tr	4.3
20:4ω6	11.8	2.1	9.2	2.5	6.6	2.3	4.8	0.5
22:4ω6	1.7	0.9	4.3	tr	3.9	0.9	4.1	tr
22:5ω6	0.9	1.3	1.4	0.7	1.8	0.6	3.8	tr
22:6ω3	4.7	1.2	3.1	tr	4.8	tr	1.3	tr

[a]Chain length: number of double bonds and position of the first double bond relative to the ω carbon atom.

[b]Not detected.

[c]Less than 0.5% of total fatty acids.

cell lipids, including the lipids contained in membranes, can be produced by varying the type of fat fed to the tumor-bearing host.

MATERIALS AND METHODS: The Ehrlich ascites tumor was grown in male CBA mice (20). These animals were placed on one of four diets when they weighed approximately 12 g and were continued on the diet until the time of sacrifice. The control diet was Rockland mouse laboratory chow® containing 4.5% fat, made up of 35% saturated, 31% monoenoic-ω9 and 30% polyenoic-ω6 fatty acids. The two test diets were standard mixtures prepared by Teklad Mills, Chagrin Falls, Ohio, to which were added either 16% coconut oil (87% saturated fatty acids) or sunflower oil (58% polyenoic-ω6 fatty acids). As a further control, a fourth diet containing only the fat-free standard mixture also was employed. Groups of mice were inoculated with the tumor 16, 30 or 50 days after being placed on one of the diets, and the tumors were harvested 12 days after transplantation.

The tumor cells were separated from the ascites plasma and washed (20). Lipids were extracted with a chloroform-methanol mixture (21) and separated by silicic acid column chromatography (22). After saponification and methylation (23), the fatty acid methyl esters were separated by gas-liquid chromatography using a 6' x ¼" column containing 10% Silar 10C on Gas-chrom Q (100-120 mesh). Peaks on the chromatogram were assigned according to standards obtained from Applied Science Laboratories. Areas under the peaks were determined using a Hewlett-Packard calculator, model no. 9810A, equipped with a digitizer and programmed for determination of an area within an enclosed figure.

RESULTS: Table I shows that considerable changes were produced in the fatty acid composition of the polar and neutral lipids of the Ehrlich ascites cells when the type of fat fed to the tumor-bearing mouse was varied. As compared with cells grown in mice fed regular chow, those from mice fed coconut oil had much higher contents of ω9-monoenoic fatty acids and much lower amounts of the ω6- and ω3-polyenoic acids. By contrast, cells obtained from mice fed sunflower oil had higher levels of saturated and ω6-polyenoic acids and lower levels of ω9-monoenoic and ω3-polyenoic acids. Cells obtained from animals fed the fat deficient diet contained large amounts of ω9-monoenoic acids and very little ω6-polyenoic acids. These "fat deficient" changes are even larger than those produced in Ehrlich cells by Bailey and Dunbar (24).

Fig. 1 illustrates the fatty acids contained in the polar lipids of Ehrlich cell membrane fractions. The differences in membrane fatty acyl group composition reflect those observed in the polar lipid fractions of the corresponding intact cells (Table I).

In additional experiments, we found that the lipids of the tumor plasma

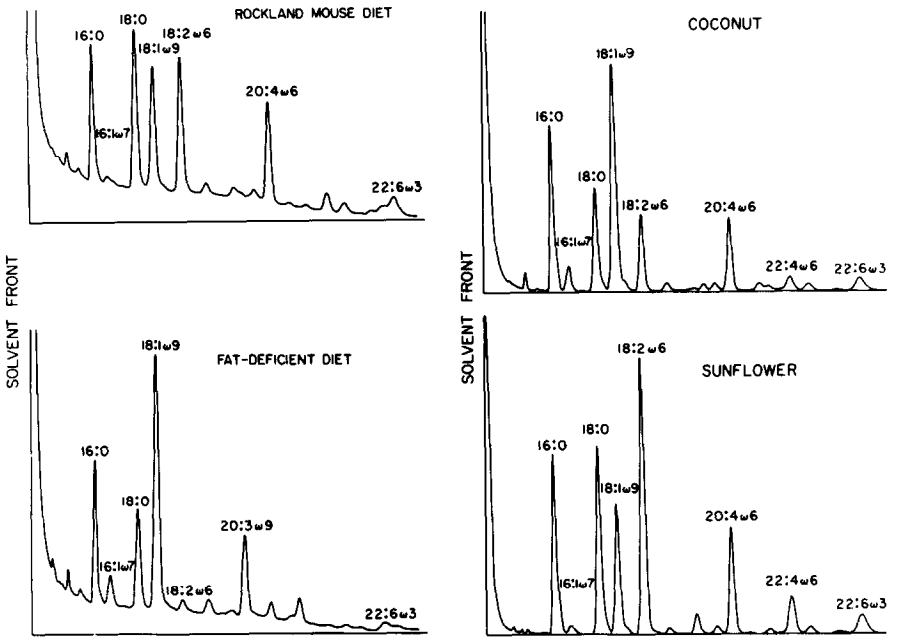

Fig. 1 Gas-liquid chromatograms of the fatty acids in membrane polar lipids. The Ehrlich cells were grown in mice fed the four different diets. The cells were ruptured by nitrogen cavitation, and a membrane fraction was isolated from the homogenate between 16,000 and 105,000 x g.

lipoproteins and liver from the tumor-bearing mouse exhibited changes in fatty acid composition similar to those occurring in the tumor cells. We also observed that the changes in fatty acid composition of tumors grown in mice that were on a special diet for only 16 or 30 days were similar but not as large as when the mice were fed for 50 days prior to tumor inoculation.

DISCUSSION: This system provides a model for investigating the relationships between lipid structure, membrane properties and cell function in a rapidly growing tumor. The system even offers some advantages for studies of this type concerning mammalian cells in general. Ehrlich cells can be produced in extremely large amounts at only a fraction of the effort and cost required to maintain cells in long-term culture. Therefore, we have found this system to be especially useful for experiments requiring the preparation of large quantities of plasma membranes from a mammalian cell suspension.

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