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Biological Optimization of Hyperthermia: Modification of Tumor Membrane Lipids*

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Abstract—*The sensitivity of the solid mammary adenocarcinomas CA755 and MtGB, grown in the medial aspect of the hind legs of host mice, to local hyperthermia* (43.5 ± 0.1°C *for* 1 hr) *was increased by feeding the host mice a diet enriched in linoleic acid. The enhanced sensitivity was expressed only when the diet was fed for* 15 days *prior to the tumor transfer. Infusion of lidocaine into the tumor immediately before the hyperthermic exposure enhanced the thermal sensitivity of the controls but not of the linoleic acid-enriched tumors. Sensitivity was analyzed by tumor growth rates and growth delay following exposure. The fatty acid patterns revealed that the proportion of polyunsaturated fatty acids* (20:4 *and* 22:6) *decreased reciprocally with increased linoleic acid in the liver phospholipids, whereas in the tumor all polyunsaturated fatty acids increased at the expense of monounsaturated fatty acids. These studies suggest that dietary lipids affected tumor cell sensitivity to hyperthermia.*

INTRODUCTION

HYPERTHERMIA was first considered to have therapeutic value as a treatment for cancer in the mid-19th century [1]. More recently, hyperthermia has received renewed attention as a means of treatment, especially when used in conjunction with other agents such as radiation, chemotherapy [2–5] and local anesthetics [6, 7]. The mechanism underlying the synergism between hyperthermia and these agents is unknown; indeed, the mechanism for tumor regression due to hyperthermia alone is unknown. Nevertheless, a considerable amount of evidence suggests that the mechanism of hyperthermic killing involves damage to cellular membranes which serve central roles in maintaining cellular activities. Based on earlier work with *E. coli*, we postulated that a primary determinant of hyperthermic sensitivity is membrane microviscosity [8, 9]. It is reasonable to presume that heat-induced alterations in physical properties of the membrane lipid matrix lead in turn to changes in protein–lipid interactions [10]. Such disruptions could modify the energetics of the cell and other membrane processes associated with cell homeostasis. When such disruptions are large enough or exist for sufficient time, the cell loses its ability to reproduce [8]. Treatment of mammalian cells with anesthetics, which are membrane-active agents [6], or with alcohol [11] increase cell killing following hyperthermic treatment *in vivo* and *in vitro*. Surface membrane alterations in mammalian cells have also been reported [12–14] and indirectly implicate membrane damage in the pathogenesis of cell inactivation following hyperthermia.

A hyperthermic therapeutic enhancement could be realized if tumor cell membranes were either naturally more fluid or could be made so [8]. Dietary modification has been used to alter the membrane lipid composition, and thereby the membrane microviscosity, of murine L1210 cells [15]. We have modified the membrane lipid composition of murine P388 ascites cells by feeding host mice diets high in either saturated or unsaturated fatty acids [16]. Estimates of hyperthermic cell killing indicate that cell killing was significantly greater in cells grown in animals fed highly unsaturated fatty acid diets [16].

Surface alterations of similarly grown and heated P388 cells were studied by scanning

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electron microscopy [14] and found to be correlated with membrane fatty acid composition. Cells high in saturated fatty acids responded differently to hyperthermic treatment *in vitro* than cells high in unsaturated fatty acids (UFA). The morphological response of highly saturated cells modified by procaine resembled heated cells obtained from animals fed a diet high in UFA [15].

In this study we report results with two solid mouse mammary tumors in which their thermosensitivity was markedly modified by their lipid fatty acid composition. It represents an extension of our ongoing efforts to modulate the response of cells to hyperthermia by nutritional means.

MATERIALS AND METHODS

Diet and animals

The fat-free test diet fortified with vitamins A, D, E and biotin* was mixed (84:16) with a fat high in- (safflower oil, 76.3%),† low in- (beef tallow, 3.4%),‡ devoid of- (hydrogenated coconut oil)* or with linoleic acid.* The mixed diet contained 49.1% sucrose, 17.7% purified casein, 16% fat, 13.2% cellulose, 3.4% USP XIV mix and 0.6% vitamin mix.* A commercial, cereal-based stock diet§ served as the control diet. Weanling male BDF1 and C3H mice, housed 5 to a cage, were fed the stock diet *ad libitum* prior to the experimental studies. The fat-modified diets were given to the mice either on the day of or 2–3 weeks prior to the day of tumor pass. Control mice were continued on the stock diet regimen.

Tumors

The mouse mammary adenocarcinoma CA755 and mammary carcinoma MtGB were grown respectively in donor BDF1 and C3H mice. After cervical dislocation, the solid tumor tissue was washed, minced and passed with minimal essential medium (MEM) containing antibiotics through a Snell cytosieve. After centrifugation and suspension (10%) in MEM the cells were injected (0.05 ml) subcutaneously in the medical aspect of the host's hind leg. Tumor size after treatment was determined on alternate days. The major and minor axes of the tumors were measured with vernier calipers and tumor volume indices (TVI), assuming an ellipsoidal shape, were calculated (mm^3).

The individual TVI were plotted on semilogarithmic graph paper as functions of time. From these lines mean estimates were made of growth delay (T_d), doubling time ($T_{2\times}$) and time required for growth to 2000 TVI (T_{2000}). The unit for each parameter is days ± standard deviation ($n \geqslant 10$).

Hyperthermia

The mice were anesthetized (14 mg i.p. chloral-hydrate) and placed on carriers which permitted the tumor-bearing leg to be immersed for one hour into water while permitting normal blood flow. The water bath, regulated by a Tecam TU-14 temperature control unit, was maintained at 43.5 ± 0.1°C under a cover of 2-cm polyethylene spheres. The air temperature above the cover never exceeded 37°C. The tumors were exposed to hyperthermia seven days after implantation when the TVIs ranged between 80 and 200. When indicated by the experimental design, lidocaine was infused by injection (2 mg/0.05 ml saline) into three areas of the tumor 5 min before starting the heat treatment.

Lipid analysis

Methyl esters of the fatty acids in the phospholipids isolated by thin-layer chromatography on silica plates from the lipid extracts of livers and tumors were prepared according to standard transesterification techniques using 2.5% HCl in methanol at 70–75°C for 2 hr. The fatty acid patterns were determined by chromatography on a 10% DEGS column with a temperature program (3°C/min from 150 to 190°C). The model 5730 A Hewlett-Packard gas chromatograph was coupled with an H-P Model 3380 A integrator. Retention times of standardized preparation of fatty acid methyl esters served as the basis for constructing semilogarithmic plots to yield equivalent chain length for the identification of the phospholipid fatty acids. From these data the mean chain length (MCL) and unsaturation index (UI) were calculated as follows:

$$\mathrm{MCL} = \frac{\Sigma\, 16\times(\%\ 16{:}0+16{:}1)+18\times(\%\ 18{:}0+18{:}1+18{:}2)+20\times(\%\ 20{:}4)+22\times(\%\ 22{:}6)}{100}$$

$$\mathrm{UI} = \frac{\Sigma\, 0\times(\%\ 16{:}0+18{:}0)+1\times(\%\ 16{:}1+18{:}1)+2\times(\%\ 18{:}2)+4\times(\%\ 20{:}4)+6\times(\%\ 22{:}6)}{100}$$

RESULTS

Mice fed the linoleic acid diet failed to thrive; all other dietary regimens supported essentially equal rates of growth both for the BDF1 hosts and the CA755 tumors. The growth characteristics of tumors reared in mice fed the safflower oil diets

*Teklad Test Diets, Madison, WI (catalog No. 170293).
†Pacific Vegetable Oil International, Inc., Richmond, CA.
‡Oscar Mayer and Co., Madison, WI.
§Purina Lab Chow, Ralston Purina, St. Louis, MO.

were indistinguishable from those of tumors reared in chow-fed hosts. The $T_{2\times}$ and T_{2000} were 1.8 ± 0.3 and 14.7 ± 2.2 respectively. Likewise, following exposure to hyperthermia, the T_{2000} of tumors reared in animals fed the safflower oil diet beginning on the day of tumor pass was not distinguished from that of tumors reared in chow-fed hosts. The respective T_ds, 6.1 ± 2.9 and 4.9 ± 2.6 (Fig. 1), showed that this dietary regimen failed to alter the tumor's response to hyperthermia. The lidocaine treatment enhanced the sensitivity of each group of tumors to hyperthermia; T_ds were increased to 10.4 ± 3.4 and 10.1 ± 3.6 (Fig. 1).

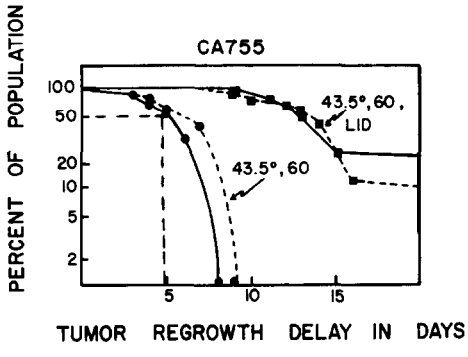

Fig. 1. The influences of the dietary regimen and of lidocaine adjunct on the T_d *response to the hyperthermic modality. The safflower oil diet was introduced on the day of tumor pass. The* T_d (---) *was* 6.1 ± 2.9 *for this group and for the control* (——) 4.9 ± 2.6. *The lidocaine adjunct increased the respective* T_d *to* 10.4 ± 3.4 *and* 10.1 ± 3.6. *No tumor regrowth occurred in* 2 *of* 12 *control and* 1 *of* 12 *test animals exposed to the combined modalities. The* T_d *represents the time when* 50% *of the tumors still did not begin to regrow as indicated by the dashed ordinate lines.*

Similar results, namely that the dietary treatment failed to change the tumor's sensitivity to hyperthermia, were forthcoming from all experiments except that in which the hosts were fed the safflower oil diet for at least 2 weeks prior to the day of tumor pass. In these hosts the tumors developed in parallel with the tumors reared in the controls (Fig. 2). The T_{2000}s respectively were 13.8 ± 1.6 and 13.1 ± 2.4 and the $T_{2\times}$s, 1.6 ± 0.4 and 1.8 ± 0.3. Following exposure to hyperthermia, the control tumors experienced a mean T_d of 5.8 ± 3.4 whereas that of the test tumors was 14.6 ± 6.3 (Figs 2 and 3). The respective T_{2000}s were 19.1 ± 2.9 and 27.5 ± 7.0 (Fig. 2). The lidocaine infusion increased the thermal sensitivity of the tumors grown in the chow-fed group. The T_d increased from 5.8 ± 3.4 to 10.6 ± 2.2. The anesthetic agent had little effect on the thermal sensitivity of tumors reared in the safflower-fed animals (Fig. 3). The size of the tumor played a major role in determining the level of enhancement of thermal sensitivity that was introduced by the dietary regimen. Diet did not affect tumor growth delay when TVIs ranged between 120 and 150 at the time the tumors were exposed to hyperthermia. The T_ds of the control (6.5 ± 5.9) and of the pre-fed safflower (5.0 ± 4.6) groups were similar.

A relationship between tumor size and sensitivity to hyperthermia was also demonstrated in studies of MtGB tumors. Tumors were reared in chow-fed hosts and in hosts pre-fed the safflower oil diet for 15 days. Control (TVI 173) and test (TVI 173) tumors were exposed to hyperthermia; the T_d of the control tumor was

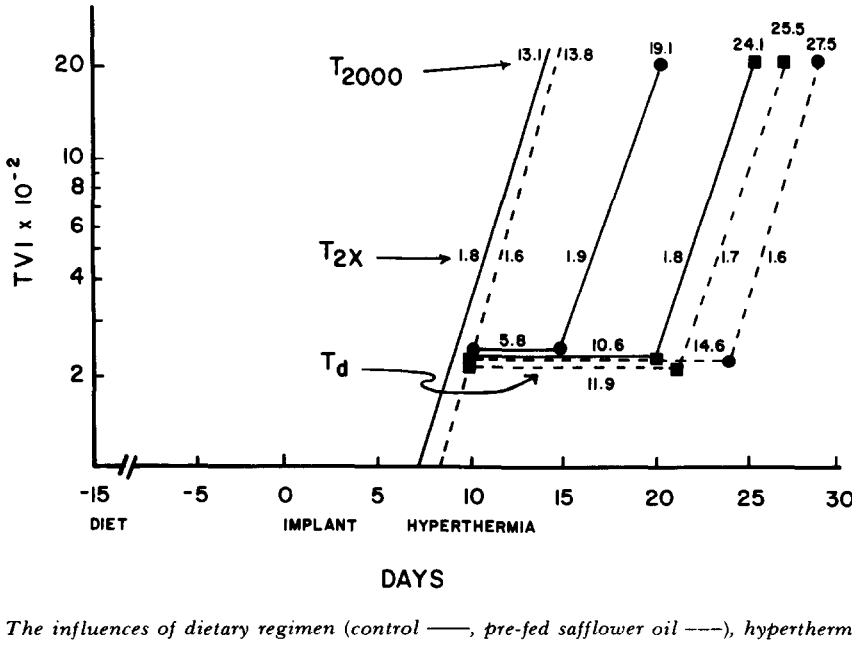

Fig. 2. The influences of dietary regimen (control ——, pre-fed safflower oil ---), hyperthermia (•) and combined hyperthermia-lidocaine modalities (■) on CA755 tumor growth and regrowth.

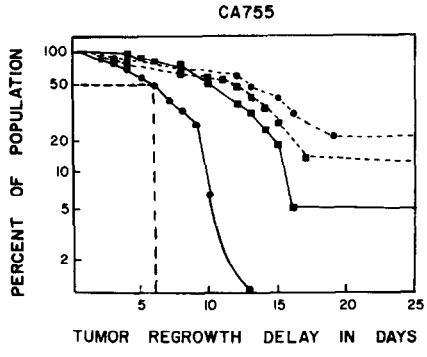

Fig. 3. The influence of the dietary regimen (control ——, pre-fed safflower oil ---), hyperthermia (•) and combined hyperthermia-lidocaine modalities (■) on the T_d response of CA755 tumors. No tumor regrowth occurred in 3 of 15 (•——•, 1 of 18 (■——■) and 2 of 17 (■----■) experimental hosts. The T_ds are: •——• 5.8 ± 3.4; ■——■ 10.6 ± 2.2; •——• 14.6 ± 6.3; and ■---■ 11.9 ± 4.1. T_d calculated as indicated in Fig. 1.

3.2 ± 2.1 and of the test tumor, 6.2 ± 4.3 (Fig. 4). In a repeat of this experiment the tumors reared in the chow-fed mice grew more slowly than the test tumors. In this study the test tumor had a TVI of 196 and the T_d was 6.4 ± 3.2. When lidocaine was used the test tumor had a TVI of 214 and a T_d of 7.3 ± 2.1. The control tumors injected with lidocaine and exposed at TVIs of 86 and 110 were more sensitive to hyperthermia (Fig. 4). The addition of lidocaine approximately doubled the T_d from 5.4 ± 4.1 to 9.9 ± 3.1. The lidocaine adjunct enhanced the thermal sensitivity of the chow group tumors but not that of the safflower oil group.

The fatty acid profiles obtained from the phospholipids extracted from both the control and test host livers and from the tumors (TVI 150) are given in Table 1. The tumor phospholipid fatty acid profile was distinguished, within a dietary group, by fatty acids of shorter MCL and of a lower UI. These differences in the above-described parameters can be traced to the higher polyene content of the liver phospholipids and the higher monoene content of the tumor phospholipids. The dietary impact on the liver phospholipid fatty acid pattern (and kidney and spleen; data not shown) was in the polyene component. The safflower oil diet elevated the diene and tetraene content and markedly lowered the hexaene content of the liver phospholipids. These changes resulted in a lowering of both the MCL and the UI of the fatty acids. This accommodation of the high dietary linoleate intake was not observed in the tumor phospholipids; overall the polyene content of these lipids increased with a reciprocal decrease in the monoenes.

The more thermo-sensitive CA755 tumor and the BDF1 host liver phospholipids contained an unidentified fatty acid, the quantity of which was elevated by feeding the safflower oil diet. These tumor phospholipids, containing higher proportions of the tetraene and hexaene, had an overall higher MCL and UI than did the MtGB tumors.

In both tumor lines the TVI was shown to play an important role in determining the relative sensitivity of control and test tumors. In Fig. 5 the UI, MCL and abbreviated fatty acid profiles are plotted against the TVI of MtGB tumors reared in

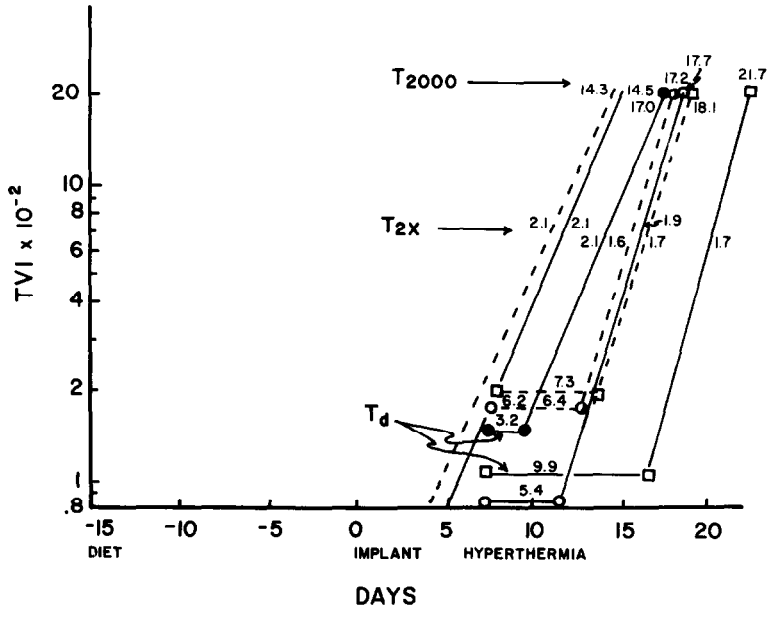

Fig. 4. The influences of dietary regimen (control ——, pre-fed safflower oil ---), hyperthermia (•,○) and combined hyperthermia-lidocaine modalities (□) on MtGB tumor growth and regrowth. Closed symbols are calculated from experiment 1 and open from experiment 2. Open/closed symbols are data from experiments 1 and 2 which fall on the same point and thus must be represented by one symbol because of overlap.

Table 1. Fatty acid patterns of liver and tumor phospholipids of mice fed stock and safflower oil-modified diet

	Chow				Safflower oil-modified			
	BDF_1		C_3H		BDF_1		C_3H	
Fatty acid	Liver	CA755	Liver	MtGB	Liver	CA755	Liver	MtGB
16:0	20.9	20.7	18.9	23.0	19.4	21.7	14.8	23.6
16:1	1.0	3.7	1.2	3.6	0.3	0.8	—	2.9
18:0	17.4	19.3	19.1	18.6	20.5	20.4	19.4	16.5
18:1	9.0	15.5	8.4	23.2	5.8	8.8	6.4	15.9
18:2	12.5	7.4	11.0	10.7	20.2	10.0	22.2	18.6
20:4	17.9	19.1	20.1	12.5	24.0	22.6	33.4	15.4
22:6	18.9	7.8	21.3	6.5	3.8	7.7	3.7	5.3
—	2.3*	6.0*	—	—	6.1*	8.7*	—	—
MCL	18.7*	18.1*	18.8	17.6	18.3	18.5	18.5	17.7
UI	2.3*	1.7*	2.4	1.4	1.8*	1.8*	2.1	1.5
% PUFA	49.5(50.7)*	34.3(36.5)*	52.4	29.7	48.0(51.1)*	40.3(44.1)*	59.3	39.3
% MUFA	10.0(10.2)*	19.2(20.4)*	9.6	26.8	6.1(6.5)*	9.6(10.5)*	6.4	18.8
% SFA	38.3(39.2)*	50.0(42.6)*	38.0	41.6	39.9(42.5)*	42.1(46.1)*	34.2	40.1
P/S	1.29	0.86	1.38	0.71	1.20	0.96	1.73	0.98

*Fatty acid excluded from calculations.

hosts fed the chow or safflower oil diet. The smaller tumors share in common a UI of 1.0, an MCL of 17.5 and a fatty acid profile of 25% saturated, 45% monounsaturated and 30% polyunsaturated fatty acids. The small tumors also share equal thermal sensitivity. With an increase in the TVI the proportions of saturated and polyunsaturated fatty acids increase; in those tumors reared in chow-fed hosts the increase in these acids was associated with parallel decreases in mono- and diunsaturated fatty acids. In those tumors reared in the safflower oil-fed hosts the proportion of the polyunsaturated fatty acids increased in parallel with that of the saturated species. The proportion of unsaturated to saturated fatty acids decreases in a consistent fashion between groups with increasing TVI.

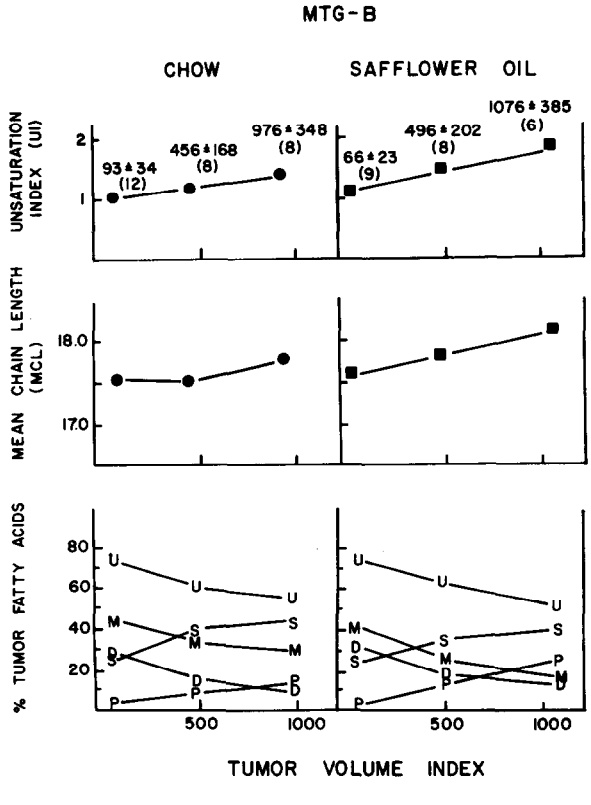

Fig. 5. Changes in the phospholipid fatty acid characteristics with increase in TVI. S, palmitic and stearic acids; M, palmitoleic acids; D, linoleic acid; P, arachidonic and dodecahexaenoic acid; U (M + D + P).

Examination of the data reveals a major discrepancy between the two groups in the ratio of polyunsaturated (P) to saturated fatty acids (S). With increases in TVI the ratio in the chow group tumors increases from 0.1 to 0.35 and in the safflower oil tumors from 0.1 to 0.7. The double bonds falling between the Δ9 carbon and the ester bond of the phospholipid confers disorganization or unstability to the lipid bilayer, whereas acyl chains saturated in this region contribute stability to this paraffin boundary of the bilayer [17, 18]. Membrane fluidizing agents, e.g. lidocaine, therefore exert a more disruptive force on membranes composed of more saturated acyl chains. As shown in Figs 2–4, the lidocaine adjunct was effective in those tumors reared in chow-fed hosts, tumors which contained the lesser content of the paraffin boundary-disrupting arachidonic and docosahexaenoic acids. The estimates of fluidity using DPH in the MtGB tumor were P 0.162 (control) and P 0.161 (safflower) and for the CA755 tumor P 0.213 (control) and P 0.212 (safflower). This was confirmed using a different probe, ANS at 30°C, which yielded a value of P 0.255 for the control and P 0.250 for the treatment groups.

DISCUSSION

We have demonstrated that the thermal sensitivity of solid tumors, like that previously

recorded for ascites cells, can be enhanced through a diet-mediated modification of the tumor lipids [16]. The dietary treatment involves a somewhat exaggerated intake of linoleic acid, e.g. 26% of total energy. The modification of the tumor phospholipid fatty acid pattern yielding modest increases in the UI and MCL traces to a major decrease in the monoene fraction (oleic and palmitoleic acids) and corresponding increases in the linoleic and arachidonic acid fractions. The saturated fatty acid (palmitic and stearic acids) fraction was not influenced by the dietary treatment. The relatively modest changes in fatty acid content in the solid tumor we studied had little effect on estimates of plasma membrane fluidity at 30°C using DPH. Using ascites cells grown in mice fed diets differing in levels of unsaturation, however, others did find differences in membrane fluidity [15]. In less complex bacterial systems grown in selected lipid media, shifts in fluidity were also found [8, 9].

Whereas the capacity of the tumor cells to respond to the modification of dietary lipids was limited, the response of host liver was conspicuous. In both the BDF1 and C3H host livers, the dietary impact was to elevate the linoleic and arachidonic acid fraction and to precipitously decrease the docosahexaenoic acid fraction of the hepatic phospholipids. These shifts underlie marked decreases in the UI and MCL. The microviscosity estimate of the BDF1 liver plasma membrane, measured at 30°C using ANS, was decreased slightly. The P-values dropped from 0.271 to 0.261. The influence of these changes in membrane composition on fluidity was not determined at physiological or hyperthermia exposure temperatures. However, the results gained using the lidocaine adjunct suggest that the fluidization of the cellular membranes of tumors reared in the safflower oil-fed hosts was maximized by the dietary modification; fluidization, presumably correlated with thermal sensitivity, was increased by the lidocaine adjunct in the tumors reared in chow-fed hosts. Moreover, the diet-mediated changes in the fatty acid characteristics of the CA755 tumor phospholipids were of greater magnitude than were found in the MtGB tumor. The modification of the former tumor provided the greatest enhancement of thermal sensitivity.

This relationship between phospholipid fatty acid composition and thermal sensitivity was also noted in the MtGB tumors. In the smaller tumors there was no measurable difference in fatty acid composition between tumors reared in either chow-fed hosts or in safflower oil-fed hosts. With growth, however, the dietary impact on the tumor phospholipid fatty acid patterns and on thermal sensitivity became manifest.

Dietary manipulation of normal and tumor cell membrane phospholipids may have important therapeutic implications. The response of the liver in reducing synthesis of dodecahexaenoic acid (22:6) in animals fed a diet high in linoleic acid (18:2) would tend to increase its resistance to a hyperthermic insult. In contrast, tumors growing in animals fed safflower diets are apparently incapable of manifesting any control. If such is the case, it is not inconceivable that manipulation of dietary lipids could result in improved therapeutic ratios in situations where whole-body hyperthermia is employed.

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