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# Increasing the Thermosensitivity of a Mammary Tumor (CA755) Through Dietary Modification¶

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**Abstract**—*Disruption of the integrity of tumor cellular membranes has been proposed as an initiating event in hyperthermic cell death. Thermosensitivity measured by the shift in the harmonic mean of tumor regrowth delay of CA755 mammary adenocarcinomas grown in the hind legs of male $BDF_1$ mice increased 22% when the hosts were fed a diet enriched in polyunsaturated fatty acids. Although the diet elicited the anticipated increase in tumor membrane phospholipid polyunsaturated fatty acids, the proportion of total unsaturated fatty acids decreased and the proportion of membrane-rigidifying saturated fatty acids increased. Concomitantly, the concentrations of cholesterol and phospholipid phosphorus increased and the ratio of phosphatidylethanolamine to phosphatidylcholine decreased, presumably to counter the effect of the change in the fatty acid pattern. In host liver membranes, the diet-mediated increase in proportion of polyunsaturated fatty acids was not accompanied by an increase in the proportion of rigidifying saturated fatty acids. Instead, the homeoviscous adaptation consisted of decreases in monounsaturated fatty acids and cholesterol concentration and an increase in the phosphatidylethanolamine–phosphatidylcholine ratio.*

*Addition of a natural inhibitor of cholesterol biosynthesis to the polyunsaturated fatty acid enriched-diet reversed the diet-mediated increase in the phosphatidylethanolamine–phosphatidylcholine ratio of host liver membranes. Tumor membrane lipids from hosts fed the combined dietary factors were characterized by the forementioned rigidifying increase in saturated fatty acids and compensatory decrease in the phosphatidylethanolamine–phosphatidylcholine ratio. The inhibitor reversed the compensatory increases in cholesterol and phospholipid phosphorus concentrations. As a consequence the thermosensitivity of tumors bearing this perturbed membrane was increased.*

## INTRODUCTION

BASED on studies of *E. coli* fatty acid auxotrophs grown on media enriched in specific fatty acids [1–4] we postulated that the capacity for maintaining membrane homeoviscosity is the primary determinant of thermosensitivity. Membranes of cells reflect the nature of the fatty acids available to them [5–12]. In animals, the capacity to maintain membrane homeoviscosity rests on interactions between phospholipid fatty acyl chains and cholesterol within the lipoid core and between the polar groups of the phospholipids and cholesterol at the polar surface [13, 14]. Cholesterol, being rigid in the region of its rings and flexible in its side chain, is an amphipathic molecule which exhibits dual roles with regard to the physical characteristics of membranes. In the bilayer, the bonding of the cholesterol hydroxyl group with the phospholipid polar groups orients the molecule so that its rigidity and flexibility are manifested within the sterol stiff and pliant chain regions respectively of the lipoid core [15]. Diet-mediated changes in the fatty acid composition of cellular membrane phospholipids induce compensatory changes in the cholesterol concentration [16] and in the phospholipid polar headgroup pattern [17]. A diet-mediated elevation of the polyunsaturated fatty acid content of hepatic cellular membranes is compensated by a decrease in the concentration of cholesterol [15, 16], perhaps because the removal of its side chain from the pliant chain region more effectively confers stability than does the addition of its rings to the sterol-stiff region.

Cellular membranes of tumors differ from normal membranes in having more saturated phospholipid fatty acids [18] and a 2–3-fold enriched cholesterol content [19]. Factors which alter the properties of tumor membrane influence tumor

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thermosensitivity. For example, the thermosensitivity of tumor cells is increased by the adjunctive use of membrane-fluidizing anesthetics [8, 12, 20]. Tumors containing membranes enriched in linoleic acid (metastatic vs non-metastatic [21] and diet-modified [8–11]) are more sensitive to hyperthermia. On the other hand, the cholesterol content of a series of cells is reported to be directly related to their survival following hyperthermia [22].

We postulated that a tumor containing membranes enriched in polyunsaturated fatty acids and depleted of cholesterol would be highly thermosensitive. Here we report that a diet-mediated increase in the polyunsaturated fatty acid content of CA755 tumor membranes is accompanied by major shifts in the phospholipid headgroup pattern and saturated fatty acids and increases in the cholesterol and phospholipid phosphorus concentrations. We have isolated materials from barley (Inh) which inhibit cholesterol biosynthesis [23, 24]. Feeding the Inh prevents the compensatory increase in cholesterol and as postulated, markedly enhances thermosensitivity.

## MATERIALS AND METHODS

*Animals, diets and treatments*

Weanling male BDF1 mice (Harlan Sprague–Dawley, Madison, WI) were housed five to a cage in a room with a 12 hr dark/light cycle maintained by the Animal Care Unit of the Wisconsin Clinical Cancer Center, University of Wisconsin, Madison, WI. A fat-free, cholesterol-free test diet fortified with vitamins A, D, E and biotin (catalog no. 170293, Teklad Test Diets, Madison, WI) was mixed either 90 : 10 or 84 : 16 with a fat high (safflower oil — 76.3% linoleate, SO) or adequate (beef tallow, 3.4% linoleate, BT) in linoleic acid content. The 10% fat diets (3.76 kcal/g) consisted also of 19.0% purified casein, 52.6% sucrose, 14.2% cellulose, 3.6% USP XIV mineral mix and 0.6% Teklad vitamin mix. The 16% fat diets (4.11 kcal/g) contained, in addition to fat, 17.7% purified casein, 49.1% sucrose, 13.2% cellulose, 3.4% USP XIV mineral mix and 0.6% Teklad vitamin mix. The diets were further modified, as specified in the experimental design, with the addition of 0.1% of the inhibitor (Inh). The diets were fed for 2–3 weeks prior to and for 1 week following tumor passes. Tumor implantation was carried out by injecting 0.05 ml of a suspension of the mammary adenocarcinoma CA755, grown in donor BDF1 mice, into the medial aspect of the host hind leg [8]. The major and minor axes of the tumors were measured with vernier calipers. The tumor volume indices ($mm^3$) (TVI) were calculated, assuming an ellipsoidal shape, as the product of the square of the minor and major axes. When the tumors had grown to TVI of 80–200 (6–9 days post implantation), they were exposed to hyperthermia [8]. The mice were anesthetized (14 mg i.p. chloralhydrate) and placed on carriers which permitted the tumor-bearing leg to be immersed for 1 hr into water while permitting normal blood flow. The water bath, regulated by a temperature control unit (Tecam TU-14), was maintained at 43.5 ± 0.1°C or 44.0 ± 0.1°C under a cover of 2 cm polyethylene spheres. The air temperature above the cover never exceeded 37°C. Following this exposure, the host mice were fed the respective Inh-free fat-modified diets and TVI of the tumors were estimated at 2-day intervals. The TVI for each tumor were plotted on semi-logarithmic graph paper as a function of time. Mean estimates of tumor regrowth delay (Td) were calculated from these plots.

*Tissue preparation*

When indicated by the experimental design, mice were sacrificed and samples of blood, liver and tumor obtained. Blood was obtained by heart puncture and serum prepared by centrifugation at 4500 ***g*** for 10 min. Liver and tumors were excised, washed and weighed. The tissues were suspended (1 : 2) in phosphate buffered saline (PBS, pH 7.4) and homogenates of livers were prepared by Polytron homogenization and of tumors by sonication. The 'cell membrane' fractions were prepared by centrifuging the homogenates at 20,000 ***g*** for 15 min to pellet unbroken cells and cell debris. The supernates were then centrifuged at 105,000 ***g*** for 60 min. The pellet (plasma membrane and endoplasmic reticulum) fraction was resuspended in 3 ml of PBS.

*Lipid analysis*

Tissue lipids were extracted as described by Folch *et al.* [25], and the fatty acid patterns of the extracted lipids determined as described previously [8]. Serum cholesterol was analyzed by the enzymatic method of Allain *et al.* [26] while tissue cholesterol (free + esterified) content was determined by the method of Carlson and Goldfarb [27]. Total phospholipid phosphorus (PL Pi) was determined according to the procedure of Jacobson and Yatvin [28] and the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) contents of tissue lipid extracts were separated and quantitated by the high performance liquid chromatography techniques of Chen and Kou [29].

*Protein*

Protein concentrations of tissue homogenates and membrane fractions were determined by either the biuret [30] or the Lowry [31] method using bovine serum albumin as standard.

## EXPERIMENTAL DESIGN

*Experiment* 1

Four groups of 60 mice were fed diets modified with 10% BT, 10% BTI, 10% SO or 10% SOI for 2 weeks. Liver and blood serum samples were collected from eight mice in each group. Tumors were passed to the remaining mice. After 6 days, liver, blood and tumor samples were collected from 12 mice in each group. Twenty mice per group were exposed to hyperthermia at 43.5°C; tumor development in these and in the 20 non-heated mice of each dietary group was followed to the animal death.

*Experiment* 2

Four groups of 65 mice each were fed diets modified with 16% BT, 16% BTI, 16% SO or 16% SOI for 3 weeks. Tumors were passed. Tumor growth was monitored. After the tumors had grown to a TVI of 200±50, 20 mice from each group were exposed to hyperthermia at 43.5°C, 20 at 44°C and 20 mice served as non-heated controls. Tumor growth was followed to TVI 8000mm$^3$. The tumors and livers from the five tumor-bearing mice remaining in each dietary group were collected for analysis when the TVI reached 400.

*Statistical analysis*

Statistical evaluation of results involved the use of the analysis of variance (ANOVA) and/or the *t*-statistic as described by Mendenhall [32]. A modified Cox survival model [33] was used for analyzing the Td data.

## RESULTS

The objective of these studies was to increase the thermosensitivity of tumors through dietary modifications which impose limits on the tumor membrane's homeoviscotic capacity. The dietary modifications were used to alter the membrane's fatty acid pattern [5] and cholesterol content [23, 24]. Initial studies revealed that host and normal mice differ in hepatic protein concentration and fatty acid profile and in serum cholesterol level. Differences due to one dietary modification, an increase in polyunsaturated fatty acids, are more pronounced in tumors than in hepatic lipids. Further, the effect of the second dietary modification, cholesterol synthesis Inh, is modulated both by the presence of the tumor and by the dietary fat. A summary of these effects is presented as background for understanding the diet-mediated perturbations of hepatic and tumor membranes and the effect of the latter on tumor thermosensitivity.

*Influence of tumors*

Body and liver weights and liver weight per 100 g body wt were higher in host than in normal mice (Table 1). Total liver protein of host mice (251.9±10.2 mg) was the same as that of the normal mice (251.1±8.6 mg). When expressed as protein concentration, the host animal values were lower reflecting the greater liver weights (Table 2). Total hepatic cholesterol levels of host and normal mice were similar (7590±834 and 7320±732 nmol/liver respectively). Analysis of fatty acid patterns of hepatic total lipids (Table 3) of host mice yielded a mean chain length (MCL) or 18.02±0.13 and unsaturation index (UI) of 1.54±0.19. The respective normal mice values were 17.93±0.14 and 1.48±0.20. Host mice had, independent of the dietary treatments, significantly ($P < 0.025$) lower serum cholesterol levels (Table 1).

*Influence of dietary fat*

Total body, liver and tumor weights (Table 1) and liver and tumor protein concentrations (Table 2) were not influenced by the dietary fats. The effects of the highly unsaturated SO diet were manifested in a significantly lower serum cholesterol level (Table 1, $P<0.02$), a significantly lower hepatic cholesterol–protein ratio (Table 2, $P<0.05$) and a significantly higher tumor

*Table* 1. *Body, liver, tumor weights and serum cholesterol of normal and host mice fed* 10% *dietary fat*

| | | BT | BTI | SO | SOI |
|---|---|---|---|---|---|
| Body wt (g) | Normal†: | 23.0 ± 1.5* | 22.4 ± 2.7 | 24.0 ± 2.1 | 23.3 ± 1.2 |
| | Host‡: | 23.6 ± 2.1 | 23.8 ± 1.5 | 24.5 ± 1.3 | 24.2 ± 1.2 |
| Liver wts (g) | Normal: | 1.18 ± 0.1 | 1.14 ± 0.1 | 1.13 ± 0.1 | 1.12 ± 0.1 |
| | Host: | 1.28 ± 0.2 | 1.24 ± 0.1 | 1.22 ± 0.1 | 1.20 ± 0.1 |
| g/100g body wt | Normal: | 5.1 | 5.1 | 4.7 | 4.8 |
| | Host: | 5.4 | 5.2 | 5.0 | 5.0 |
| Serum cholesterol (mg/dl) | Normal: | 154.0 ± 29.3 | 145.6 ± 17.9 | 126.8 ± 27.2 | 136.7 ± 19.8 |
| | Host: | 123.6 ± 14.9 | 112.3 ± 1.0 | 103.2 ± 10.4 | 102.1 ± 20.3 |
| Tumor (CA755)§ wt (g) | | 0.25 ± 0.07 | 0.22 ± 0.05 | 0.23 ± 0.12 | 0.26 ± 0.20 |

* Mean ± S.D.; statistical statements are given in the text.
*n* values are: †normal = 8; ‡host = 12; §CA755 = 12.

*Table 2. Effect of dietary fat on the biochemical parameters in the livers of normal and host mice and transplanted CA755*

| | BT | BTI | SO | SOI |
|---|---|---|---|---|
| | | [10% Fat] | | |
| *Liver proteins* (mg/g) | | | | |
| Normal: | 220.6 ± 22.4 | 224.2 ± 27.4 | 218.6 ± 19.4 | 214.8 ± 37.4 |
| Host: | 200.8 ± 17.8 | 209.8 ± 14.5 | 207.9 ± 17.2 | 197.7 ± 10.7 |
| *Liver cholesterol* (nmol/mg protein) | | | | |
| Normal: | 32.2 ± 9.4 | 27.8 ± 4.2 | 28.0 ± 9.2 | 28.4 ± 4.9 |
| Host: | 34.0 ± 5.8 | 29.5 ± 3.0 | 27.8 ± 4.6 | 29.1 ± 2.7 |
| *CA755 protein* (mg/g) | 126.0 ± 7.5 | 123.0 ± 6.9 | 121.4 ± 15.2 | 126.1 ± 16.4 |
| CA755 cholesterol (mg/g): | 0.89 ± 0.1 | 0.93 ± 0.2 | 1.06 ± 0.3 | 0.77 ± 0.2 |
| nmol/mg protein: | 18.2 ± 1.8 | 19.8 ± 5.1 | 20.9 ± 10.7 | 15.8 ± 6.1 |
| | | [16% Fat] | | |
| *Host liver membrane* | | | | |
| Protein (mg/g): | 38.2 ± 6.3 | 59.2 ± 2.3 | 38.0 ± 9.3 | 65.5 ± 20.5 |
| Cholesterol (nmol/mg protein): | 37.0 ± 14.2 | 35.6 ± 1.3 | 25.0 ± 10.4 | 24.4 ± 1.7 |
| *CA755 membrane* | | | | |
| Protein (mg/g tumor): | 10.8 ± 0.1 | 15.0 ± 0.9 | 9.8 ± 2.8 | 19.0 ± 0.7 |
| Cholesterol (nmol/mg protein): | 69.4 ± 8.1 | 71.6 ± 16.2 | 162.2 ± 58.6 | 76.5 ± 25.7 |

*Table 3. Fatty acid patterns* of liver, host liver and CA755 total lipids of mice fed 10% fat diets*

| Fatty acid | BT | | | BTI | | | SO | | | SOI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Liver | Host Liver | CA755 | Liver | Host Liver | CA755 | Liver | Host Liver | CA755 | Liver | Host Liver | CA755 |
| 16:0 | 23.9 ± 1.1 | 23.7 ± 2.2 | 22.4 ± 0.8 | 24.1 ± 2.7 | 24.3 ± 1.3 | 21.8 ± 0.8 | 23.8 ± 1.1 | 25.2 ± 1.9 | 23.4 | 24.7 ± 1.8 | 23.4 ± 1.6 | 25.4 ± 3.6 |
| 16:1 | 4.5 ± 1.2 | 4.9 ± 0.6 | 6.4 ± 0.5 | 3.9 ± 1.7 | 3.5 ± 1.4 | 5.3 ± 1.1 | 1.4 ± 0.8 | 0.9 ± 0.7 | 3.1 | 0.4 ± 0.7 | 0.5 ± 0.8 | 2.9 ± 1.5 |
| 18:0 | 8.2 ± 2.5 | 7.2 ± 1.6 | 10.9 ± 0.6 | 9.5 ± 4.1 | 10.5 ± 3.4 | 11.0 ± 0.6 | 13.4 ± 2.5 | 14.7 ± 1.8 | 11.5 | 14.4 ± 2.4 | 15.2 ± 2.1 | 13.4 ± 2.1 |
| 18:1 | 41.0 ± 8.6 | 40.7 ± 4.9 | 40.9 ± 1.5 | 35.2 ± 11.4 | 32.4 ± 9.3 | 37.3 ± 1.6 | 11.0 ± 1.8 | 9.5 ± 1.8 | 19.2 | 9.6 ± 1.6 | 9.1 ± 2.4 | 17.2 ± 2.4 |
| 18:2 | 7.2 ± 2.1 | 7.1 ± 1.8 | 6.9 ± 0.8 | 9.1 ± 3.2 | 9.0 ± 2.7 | 8.8 ± 0.3 | 26.4 ± 3.3 | 25.2 ± 1.7 | 25.5 | 27.8 ± 3.1 | 26.0 ± 2.9 | 19.4 ± 4.8 |
| 20:3 | 1.4 ± 0.4 | 3.6 ± 2.4 | 1.6 ± 0.6 | 2.2 ± 1.3 | 2.7 ± 1.6 | 1.1 ± 0.4 | 1.2 ± 0.3 | 1.1 ± 0.5 | 0.4 | 1.2 ± 0.2 | 1.2 ± 0.1 | 0.8 ± 0.3 |
| 20:4 | 9.2 ± 3.9 | 7.1 ± 2.0 | 7.6 ± 0.2 | 10.8 ± 4.5 | 12.3 ± 4.5 | 11.1 ± 1.7 | 18.3 ± 4.4 | 18.3 ± 2.6 | 13.1 | 17.6 ± 3.2 | 18.6 ± 2.5 | 15.6 ± 2.8 |
| 22:5 | 1.0 ± 1.1 | 1.6 ± 1.7 | 0.4 ± 0.4 | 0.74 ± 0.7 | 0.5 ± 0.2 | 1.1 ± 0.4 | 1.7 ± 0.7 | 1.8 ± 0.6 | 3.6 | 1.5 ± 0.7 | 1.7 ± 0.7 | 4.8 ± 0.7 |
| 22:6 | 3.3 ± 1.0 | 4.1 ± 1.1 | 2.9 ± 1.3 | 4.3 ± 3.2 | 4.8 ± 1.9 | 2.5 ± 0.4 | 2.6 ± 1.3 | 3.3 ± 1.4 | – | 2.8 ± 1.5 | 4.3 ± 2.0 | 0.4 ± 0.8 |
| PUFA (%) | 22.4 | 23.5 | 19.4 | 27.3 | 29.3 | 24.6 | 50.4 | 49.7 | 42.8 | 50.9 | 51.8 | 41.1 |
| MUFA (%) | 45.5 | 45.6 | 47.3 | 39.1 | 35.9 | 42.6 | 12.4 | 10.4 | 22.3 | 10.0 | 9.6 | 20.1 |
| SFA (%) | 32.1 | 30.9 | 33.3 | 33.6 | 34.8 | 32.8 | 37.2 | 39.9 | 34.9 | 39.1 | 38.6 | 38.8 |
| PUFA/SFA | 0.70 | 0.76 | 0.58 | 0.81 | 0.84 | 0.75 | 1.35 | 1.24 | 1.23 | 1.30 | 1.34 | 1.06 |

*Mean ± S.D.

cholesterol-protein ratio (Table 2, $P<0.05$). Also, the phosphatidylethanolamine–phosphatidylcholine ratio (PE/PC) was lower for the tumor lipids of the SO group (Table 4). Fatty acid patterns of liver and tumor lipids are given in Table 3. Hepatic and tumor total lipids of mice fed the SO diet were enriched in the proportion of polyunsaturated fatty acids (PUFA) and depleted in the proportion of monounsaturated fatty acids (MUFA). The proportion of saturated fatty acids (SFA) in the liver total lipids was modestly increased, an increase not noted in the tumor lipids. Overall hepatic and tumor total lipids of the SO group are characterized by increased PUFA/SFA ratio (Table 3), MCL and UI (Table 4).

*Influence of the inhibitor*

Total body, liver and tumor weights (Table 1) and liver and tumor protein concentrations (Table 2) were not influenced by the Inh. When present, many of the effects of the Inh were associated with the effects of the dietary fat. A modest lowering of serum cholesterol levels (Table 1) and of the hepatic cholesterol/protein ratios (Table 2) of host and normal mice occurred when the Inh was added to the BT but not when added to the So diet. On the other hand, the Inh reversed the SO-mediated increase in the tumor cholesterol (mg/g tissue) and cholesterol/protein ratio (Table 2).

The Inh, independent of dietary fat, was associated with increases in the MCL and UI of all host liver and tumor lipids and with lower PE/PC ratios for the tumor lipids (Table 4).

*Membranes*

The dietary treatment-mediated differences in the composition of host liver and tumors are amplified in their membraneous fractions. Host livers, compared to tumors, were 4-fold richer (Table 2) in membrane protein content (membrane protein/g tissue). Proteins recovered in the membranous fractions of liver and tumors of hosts fed the Inh-free diets were 19% and 8.5% respectively of the total tissue proteins. Neither tissue total nor membrane proteins was influenced by the dietary fat. In membranes isolated from liver of host mice, the SO diet was associated with a 33% decrease in cholesterol (Table 2), with a non-significant decrease in PL Pi and with an increase in PE/PC (Table 4). The SO dietary impact on tumor membrane composition was strikingly different. Cholesterol was 2.3-fold higher, PL Pi 2.4-fold higher and PE/PC markedly decreased (Tables 2 and 4). These differences are of greater magnitude than those recorded for whole tissues. The diet fat effects on membrane phospholipid fatty acid profiles (Table 5) also reflected the effects noted for the tissue lipids (Table 3). The proportions of PUFA/MUFA/SFA in the hepatic membrane profile of the BT and SO groups were 26 : 40 : 34 and 55 : 8 : 37 respectively. Clearly, the SO diet mediated doubling in PUFA was countered only by a decrease in MUFA. The MCL of BT group membrane phospholipids, 17.94 was shorter than that of the SO group, 18.18. The respective UI were 1.33 and 1.76. Similar differences due to dietary fat were found in the tumor membrane fatty acid profiles (Table 5). The PUFA/MUFA/SFA ratios were 13 : 60 : 28 and 30 : 23 : 46 for the BT and SO groups. Here, the doubling of the PUFA was accompanied both by a decrease in MUFA and an increase in SFA. The MCL were similar as were the UI. The UI of the SO liver and tumor are of interest. The SO diet increased the UI of the total lipids of each tissue; however, when the more saturated tumor membranes were examined, the cumulative effect of the SO diet on the UI was negligible. The value of the UI as a monitor of change in membrane composition may be questioned since in the presence of a small shift in UI, 0.97 to 1.02, there occurred a major shift, 0.46 to 0.65 in the PUFA/SFA ratio.

The Inh had no influence on the tissue total protein. However, this dietary treatment altered characteristics of the membranes so that the fraction of protein sedimented with the membranous fraction of each tissue was increased by 50% (Table 2). Across tissues and diet fat groups the

*Table 4. Lipid profile of implanted CA755 and livers of host mice fed 10% (whole tissue) or 16% (membrane fraction) dietary fat*

| | BT | | BTI | | SO | | SOI | |
|---|---|---|---|---|---|---|---|---|
| | Host Liver | CA755 | Host Liver | CA755 | Host Liver | CA755 | Host Liver | CA755 |
| *Fatty Acid MCL* | | | | | | | | |
| Whole tissue: | 17.87 | 17.76 | 17.96 | 17.85 | 18.05 | 17.85 | 18.18 | 17.95 |
| Membrane: | 17.94 | 17.62 | 18.05 | 17.73 | 18.18 | 17.66 | 18.19 | 17.74 |
| *Fatty Acid UI* | | | | | | | | |
| Whole tissue: | 1.32 | 1.16 | 1.45 | 1.28 | 1.66 | 1.45 | 1.74 | 1.50 |
| Membrane: | 1.33 | 0.97 | 1.49 | 1.14 | 1.76 | 1.02 | 1.81 | 1.25 |
| *PE/PC* | | | | | | | | |
| Whole tissue: | | 0.70 | | 0.49 | | 0.57 | | 0.45 |
| Membrane: | 1.06 | 3.74 | 0.85 | 2.88 | 1.74 | 1.15 | 0.82 | 0.82 |
| *PL P, (μg/mg protein)* | | | | | | | | |
| Membrane: | 7.1 ± 4.2 | 7.0 ± 1.1 | 7.3 ± 0.4 | 8.4 ± 2.8 | 6.3 ± 1.2 | 16.9 ± 2.2 | 4.0 ± 0.1 | 7.4 ± 1.8 |

*Table 5. Fatty acid patterns of liver and tumor (CA755) membrane fractions when host mice were fed the fat-modified diets*

| | 16% Fat diets | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Fatty acid | BT | | BTI | | SO | | SOI | |
| | Liver | CA755 | Liver | CA755 | Liver | CA755 | Liver | CA755 |
| 16:0 | 23.5 | 19.4 | 23.4 | 20.6 | 21.1 | 25.0 | 22.3 | 26.0 |
| 16:1 | 3.0 | 6.9 | 2.2 | 5.3 | – | 1.1 | 1.5 | 2.7 |
| 18:0 | 10.5 | 8.2 | 11.6 | 9.1 | 15.8 | 21.4 | 14.3 | 17.1 |
| 18:1 | 37.0 | 52.6 | 30.2 | 43.8 | 8.2 | 22.2 | 8.1 | 19.7 |
| 18:2 | 6.2 | 5.7 | 9.2 | 9.0 | 28.5 | 21.1 | 26.3 | 19.9 |
| 20:3 | 5.6 | 2.8 | 4.4 | 2.4 | 1.5 | 0.3 | 1.4 | 1.0 |
| 20:4 | 10.4 | 4.4 | 14.2 | 9.8 | 20.2 | 8.9 | 19.2 | 10.1 |
| 22:5 | 0.4 | – | 0.4 | – | 2.3 | – | 2.6 | – |
| 22:6 | 3.4 | – | 4.4 | – | 2.3 | – | 4.2 | 3.3 |
| PUFA (%) | 26.0 | 12.9 | 32.6 | 21.2 | 54.9 | 30.3 | 53.8 | 34.5 |
| MUFA (%) | 40.0 | 59.5 | 32.4 | 49.1 | 8.2 | 23.3 | 9.6 | 22.4 |
| SFA (%) | 34.0 | 27.6 | 35.0 | 29.7 | 36.9 | 46.4 | 36.6 | 43.1 |
| PUFA/SFA | 0.76 | 0.46 | 0.93 | 0.71 | 1.49 | 0.65 | 1.47 | 0.80 |

Inh elicited four common responses, namely, increases in MCL, UI and PUFA/SFA ratio and a decrease in the PE/PC ratio (Table 4), none of which provides a rationale for the increase in quantity of sedimentable protein. The major effects of the Inh were the reversals of the SO-induced increases in tumor membrane cholesterol and PL Pi (Tables 2 and 4).

In summary, these data show that the responses within tumor membranes following a dietary change are regulated differently than those occurring in hepatic membranes. Within tumor membranes, a diet-mediated increase in PUFA is accompanied by increases in SFA, cholesterol and PL Pi and a decrease in the PE/PC ratio. Presumably, these changes represent homeoviscotic responses. The Inh attenuated these responses by preventing the increases in membrane cholesterol and PL Pi.

*Hyperthermia*

The effects of dietary fat type and level and of the presence of the Inh on the sensitivity of CA755 to hyperthermia are indicated by changes in Td. Since a bias in favor of a longer mean Td might be introduced into the arithmetic mean Td by the few animals which remain tumor-free during the period of observation (35 days post treatment), the impact of the treatment was also assessed using the harmonic means of Td which are biased towards animals experiencing shorter Td (Table 6). The 43.5°C Td arithmetic and harmonic means for tumors reared in hosts fed the 16% fat diets were 60±31% and 58±12% respectively longer ($P < 0.05$) than those of the 10% fat groups. The Inh also significantly ($P < 0.05$) increased these means. The effect of fat type on tumor Td was not significant.

Increasing the treatment temperature to 44.0°C increased ($P < 0.05$) the arithmetic Td by 45% (Table 6). As described above, the Inh had a marked impact on the characteristics of tumors reared in SO-fed hosts. This impact is further manifested in the 95% increase in the arithmetic Td of this group as compared to the 50% increase in Td of the BT group. A similar pattern of changes between 44.0° and 43.5°C was observed in the harmonic means (Table 6). With treatment incorporating a 16% fat level and exposure to 44°C, the harmonic mean Td of tumors reared in SO-fed hosts (6.0, with two cures) was significantly ($P < 0.05$) longer than that for the BT-fed group (4.9). A comparison of mean Td at 10% fat, 43.5°C vs 16% fat, 44.0°C confirmed the significant ($P < 0.05$) effects of temperature, level of fat and the Inh. The impact of SO on the enhancement of the tumor's sensitivity to hyperthermia previously reported [8] is evident in the harmonic mean Tds (Table 6). For clarity of presentation of the effect of the Inh, the Td data were grouped across fat level and composition (Fig. 1). The time to fixed event model we used [33] shows that the Inh increased the tumor's thermosensitivity at each of the exposure temperatures.

## DISCUSSION

The sensitivity of CA755 tumors grown in the medial aspect of the hind legs of BDF1 mice to local hyperthermia (1 hr at 43.5° or 44.0°C) was enhanced by (1) increasing the level of dietary fat from 10 to 16%, (2) increasing the proportion of linoleic acid in the dietary fat from 3.4 to 76.3%, and (3) by adding an inhibitor of endogenous cholesterol synthesis to the diet. A combination of the treatments most effectively enhanced thermosensitivity. The dietary treatments were chosen

*Table 6. Influences of dietary fat type and level, inhibitor and thermic exposure on arithmetic and harmonic mean* Td

| | BT | BTI | SO | SOI |
|---|---|---|---|---|
| 10% Fat, 43.5° | | | | |
| *n* | 19 | 20 | 20 | 20 |
| Arith. mean ± S.D. | 2.8 ± 1.6 | 4.8 ± 2.8* | 4.2 ± 5.4 | 4.9 ± 2.6 |
| Harmonic mean ± S.E.M. | 2.5 ± 0.7 | 4.1 ± 1.35 | 2.3 ± 0.7 | 3.5 ± 1.2 |
| Cures | 0 | 0 | 1 | 0 |
| 16% Fat, 43.5° | | | | |
| *n* | 20 | 20 | 19 | 18 |
| Arith. mean ± S.D. | 6.0 ± 3.7 | 7.4 ± 5.0 | 5.8 ± 3.0 | 6.6 ± 2.9 |
| Harmonic mean ± S.M. | 4.3 ± 1.4 | 5.9 ± 3.0 | 4.0 ± 5.3 | 5.5 ± 2.8 |
| Cures | 0 | 0 | 0 | 0 |
| 16% Fat, 44° | | | | |
| *n* | 20 | 20 | 18 | 18 |
| Arith mean ± S.D. | 8.3 ± 6.0† | 10.7 ± 5.8† | 8.8 ± 6.5‡ | 12.9 ± 7.6‡ |
| Harmonic mean ± S.E.M. | 4.9 ± 1.2 | 8.0 ± 3.1 | 6.0 ± 2.0 | 8.8 ± 2.4 |
| Cures | 0 | 0 | 2 | 2 |

$P < .01$ = *
$P < .05$ = †
$P < .10$ = ‡

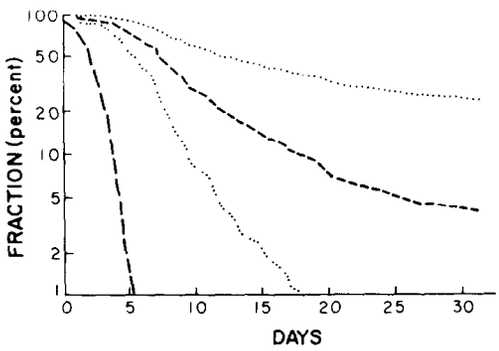


*Fig. 1. The influence of the inhibitor of cholesterol biosynthesis of tumor regrowth delay* (———, 43.5°; – – –, 44°; ··· ···, 43.5°, Inh; ·····, 44°, Inh).

specifically for their effects on lipid metabolism and hence on cell membrane lipid composition.

An interplay of lipid components is required to maintain the structural integrity of membranes. In host mice fed the linoleate-enriched SO diet, the proportion of fluidizing acids in hepatic membrane phospholipids was increased without a compensating increase in the proportion of rigidifying acids. Fluidizing fatty acids are those which introduce *cis* unsaturated bonds into the sterol stiff region, the portion of the bilayer between the phospholipid backbone at the polar surface and ubiquitous *cis* double bond at the 9 position of the unsaturated fatty acids which are esterified predominantly at the 2-position of the glycerol moiety. Fatty acids presenting only saturated bonds within this region produce rigidity. Fatty acids with *cis* double bonds only in the pliant chain region of the lipoid core also are fluidizing. In the disordered region, saturated fatty acids introduce rigidity while mono-, di- and tri-unsaturated fatty acids in which the first double bond is at the 9 position produce fluidity [15].

Compensating for the increased proportion of fluidizing fatty acids in the hepatic membranes of the SO group was a stabilizing decrease in the concentration of amphipathic cholesterol which increases fluidity within the sterol stiff region and rigidity within the pliant chain region. The final accommodation observed within the SO-conditioned membrane is a major increase in the PE/PC ratio which increases rigidity at the polar surface abutting the sterol stiff region as the result of increased electrostatic interaction.

Fatty acids of the CA755 tumor membrane phospholipids had a lower UI which was not changed by the linoleate-enriched diet. Increases in fatty acids which tend to fluidize the pliant chain region were accompanied by a compensating decrease in fatty acids with double bonds in the sterol stiff region. We postulate that the relative increase in the proportion of saturated fatty acids adds rigidity both to the sterol stiff region and to the pliant chain region. In such a situation, a compensating increase in cholesterol introduces fluidity into the lipoid core and alters the polar surface stability by substituting the less polar cholesterol OH for the repulsive effect of similarly charged phospholipids. To modulate this rigidifying influence at the polar surface, a decrease in the PE/PC ratio occurs. The disorganization of this membrane in response to the Inh-proscribed cholesterol deficit was increased both in the sterol stiff region by the increase in the proximal double bonds of the phospholipid fatty acids and on the polar surface by the perturbation of the PE/PC

ratio. We postulate that these changes, brought into play by feeding a linoleate-rich diet coupled with an inhibitor of cholesterol biosynthesis, compromise the adaptive responses resulting in a weakening of its structural integrity thereby increasing its sensitivity to heat stress.

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