Effects of Fatty Acids on Gap Junctional Communication: Possible Role in Tumor Promotion by Dietary Fat[1]

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Dietary lipids, in particular unsaturated fat, promote the development of many experimental tumors. However, no mechanisms to fully explain these effects have been elucidated. Recent reports, which we summarize here, suggest a role for gap junction-mediated intercellular communication in the process of tumor promotion. We also review tumor-promoting effects of dietary fat on experimental, particularly mammary, carcinogenesis. Our main focus is to review recent data examining the inhibitory effects of unsaturated fatty acids on metabolic cooperation in Chinese hamster V79 cells. These data suggest that inhibition of junctional communication may be involved mechanistically in the promotion of tumors by high levels of dietary unsaturated fat. Finally, potential mechanisms by which unsaturated fatty acids inhibit metabolic cooperation are examined.
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It has been suggested that environmental factors and conditions may be causally related to many cancers in humans (1,2). In recent years the nutritional aspects of cancer causation have been one of the most intriguing and extensively examined environmental factor(s) in cancer research. Epidemiological evidence has indicated that the incidence of breast cancer among women is related to many nutritional components, in particular the intake of dietary fat. Strong positive correlations between total dietary fat consumption and breast cancer incidence and death rates have been reported (3-11). Other types of cancers also have been related to the intake of dietary fat (3,4,6,8,11).

Such epidemiological evidence has been substantiated by widespread reports that high levels of dietary fat stimulate tumor development in many experimental cancer systems (for reviews, see refs. 6,12,13). The tumor-promoting capacity of high fat diets has been examined extensively. However, any mechanisms to explain these effects remains controversial. Recently, it has been suggested that unsaturated fatty acids may directly influence tumor cell growth processes by inhibiting gap junction-mediated intercellular communication (14). In our report, tumor-promoting effects of dietary fat on experimental cancer are reviewed. In addition, the effects of fatty acids on metabolic cooperation are reviewed, and the possible role inhibition of gap junction-mediated intercellular communication in high fat diet-induced tumor promotion is addressed.

EXPERIMENTAL MAMMARY TUMOR PROMOTION BY DIETARY FAT

The tumor-promoting effects of dietary fat have been well documented using many experimental mammary tumor systems. In 1942, Tannenbaum first described a relationship between dietary fat and the development of spontaneous mammary tumors in mice (15). In the 1960s and 1970s, many laboratories confirmed and extended his initial observation using many different murine mammary tumor models. Elevated levels of dietary fat consistently and significantly stimulate the development of spontaneous, carcinogen-induced and transplantable mammary cancers (Table 1; for reviews, see refs. 6,12,13). In general, animals fed high levels of dietary fat (20% by weight) develop more tumors (increased tumor multiplicity) at a higher frequency (increased tumor incidence) and more rapidly (reduced latency period for tumor appearance) than animals fed a moderate (5%) or low (0.5%) fat diet. The tumorigenesis of virtually every mammary tumor model examined is enhanced by consumption of elevated dietary fat.

TABLE 1

Experimental Mammary Tumor Models That Are Stimulated by Elevated Levels of Dietary Fat[a]

Spontaneous mammary tumors
Mice
Mammary carcinomas in DBA mice
Mammary carcinomas in C3HJ mice
Mammary carcinomas in C3H/St mice
Mammary carcinomas in C3H/Crgl mice
Mammary carcinomas in TM mice
Mammary carcinomas in obese (gold auro thio-glucose-treated) C3H/Crgl mice
Rats
Mammary fibroadenomas in Sprague-Dawley rats
Mammary carcinomas in Sprague-Dawley rats
Carcinogen-induced mammary tumors
Mice
DMBA-induced mammary carcinomas in C3H-A^{vy}fB mice
Rats
DES-induced mammary carcinomas in AxC rats
DMBA-induced mammary fibroadenomas in Sprague-Dawley rats
DMBA-induced mammary carcinomas in Sprague-Dawley rats
NMU-induced mammary carcinomas in Sprague-Dawley rats
NMU-induced mammary carcinomas in Fischer rats
NMU-induced mammary carcinomas in Long-Evans rats
AAF-induced mammary carcinomas in AES rats
X-ray-induced mammary carcinomas in Sprague-Dawley rats
Transplantable mammary tumors
Mice
BALB/c mammary carcinomas
C3H/Crgl mammary carcinomas
C3H-A^{vy}fB mammary carcinomas
Rats
DMBA-induced mammary carcinomas in Wistar/Furth rats
R3230AC mammary carcinomas in Fischer rats

[a]Compiled from refs. 6, 12 and 13.

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The promotion of cancer by high levels of dietary fat is not restricted to, nor specific for, mammary cancer. Development of other experimental cancers is also enhanced by high fat diets. High dietary fat enhances rat colon carcinogenesis induced by 7,12-dimethylbenz(*a*)anthracene (DMBA), dimethyl hydrazine (DMH) and other carcinogens (16-19). Diets with high levels of polyunsaturated fat stimulate rat pancreatic carcinogenesis induced by azaserine (20) as well as the growth of AK3T3 sarcoma cell line tumors in mice (21). Mouse tumors of the integument (22), lymphatic system (23), lung (23) and central nervous system (24) are also promoted by dietary fat.

Most reports suggest that dietary fat enhances mammary tumor development by acting at the promotional stage of the tumorigenic process. The level of fat intake after carcinogen administration ("initiation") is more important in enhancing mammary tumorigenesis than the level before (7,25). Dietary fat exhibits many characteristics of classic tumor promoters as defined by Berenblum (26), including dose-response relationships and reversibility (14,27-29). Also, when high fat dietary treatment is delayed up to 20 wk after carcinogen (DMBA) administration, increased mammary tumor development is still observed in rats fed a high fat diet (30). Treatment of rats with a high dietary fat regimen for equal (3-wk) time periods similarly promotes mammary tumor development, whether the dietary treatment is initiated 0, 2 or 4 wk after DMBA administration (14). Furthermore, Ip and Sinha (31), using explanted mammary glands treated with DMBA, demonstrated that the fat level in recipient rats' diet was more important in subsequent mammary tumor development than that in donor rats' diet. The growth enhancement of many transplantable murine mammary tumors (i.e., already transformed) by high levels of dietary fat also suggests that dietary fat acts at the growth promotional phase of tumorigenesis.

The type of dietary fat is important for the enhancement of mammary tumorigenesis by high fat diets. In general, diets high in polyunsaturated fat appear to promote experimental murine mammary tumor development more than diets high in saturated fat (32-35). Because linoleic acid was the major component of most polyunsaturated fats and oils in these studies, it has been suggested that the role of linoleate itself may be important. Spontaneous mammary tumorigenesis in C3H/St mice is enhanced by increasing the level of linoleic acid at the expense of other fatty acids in the diet (36). Also, linoleic and linolenic, but not oleic, acids appear to be important in the stimulation of DMBA-induced rat mammary tumorigenesis (37). However, Carroll reported that DMBA-induced rat mammary tumorigenesis was similar for rats fed 20% high fat diets containing primarily either oleic acid (olive oil) or linoleic acid (sunflowerseed oil) (7). Also, Dayton et al. (38) found no marked differences in the ability of diets containing high-oleic and high-linoleic safflower oil to promote DMBA-induced rat mammary tumorigenesis. The importance of unsaturation in the promotion of mammary tumorigenesis by dietary fat may be limited, since it appears that once a minimum requirement for unsaturated (essential) fatty acids is met, the total amount of fat, whether saturated or unsaturated, is what determines the influence of high fat levels (7). However, in vitro studies by Kidwell and colleagues have shown that unsaturated fatty acids enhance, whereas saturated fatty acids inhibit, the growth of normal and neoplastic rat mammary epithelium in cell culture (39-41). Also, addition of oleic, linoleic and arachidonic acids stimulates the growth of X563.5 mouse melanoma cells in vitro (42). Preliminary results from our laboratory also indicate that linoleic acid stimulates the growth of V79 Chinese hamster cells in vitro (unpublished data).

The orientation of the double bond as well as the chain length have recently been shown to have an influence on in vivo mammary tumor promotion by dietary fat. Diets with *trans*-unsaturated and with saturated fatty acids are similar in their mammary tumor-promoting capacities (43). Also, a report by Cohen et al. (44) suggests that the mammary tumor-promoting effect of high dietary fat is diminished by substitution of medium chain triglycerides for the more common long chain unsaturated triglycerides.

While the promotion of mammary tumorigenesis by high levels of dietary fat is well documented, any mechanism by which dietary fat promotes mammary tumorigenesis is unclear. Some investigators have suggested that indirect endocrine-related mechanisms (i.e., increased secretion of prolactin and/or estrogen) are involved (45-49). This concept has been reviewed and evaluated (12,13). Reports that consumption of a high fat diet alters circulating levels of prolactin and/or estrogen during certain stages of the estrous cycle have been inconsistent: both increased (47-51) and unchanged (52-55) levels have been reported. In many of these studies, hormone levels were assessed using single-point determinations during different stages of the estrous cycle as well as potentially stressful blood sampling techniques that may have influenced circulating prolactin levels. Also, the small sample size in many reports makes interpretation of observed differences difficult. Aylsworth et al. (55), using in-dwelling right atrial cannulas and repeated blood sampling techniques from "unstressed" animals during the entire estrous cycle, found no difference in circulating prolactin levels in rats fed a high (20%) or moderate (4.5%) fat diet. Indirect endocrine-related mechanisms cannot account for the promotion of mammary tumorigenesis by dietary fat for additional reasons: The development and growth of hormone (pituitary and ovarian)-unresponsive or independent mammary tumors is enhanced by high dietary fat (56-59). Also, the development and growth of many experimental tumors that do not appear to be influenced by the endocrine system are enhanced by high dietary fat (17-21), suggesting that similar mechanisms are involved that do not include the endocrine system. In addition, when circulating levels of estrogen and prolactin are controlled by endocrine and drug manipulations, high fat diets still promote mammary tumorigenesis (60). Also, when serum prolactin levels are similarly elevated in hypothalamic median eminence lesioned rats, mammary tumor development is still enhanced in animals fed a high fat diet (51).

Another proposed mechanism is an increased mammotrophic hormone responsiveness in incipient mammary gland tumor tissue. Cave and Erickson-Lucas (61) have reported that prolactin receptors in NMU-induced mammary tumors are increased in rats fed a 20.0% (high) fat diet compared to a 0.5% (low) fat diet. However, these differences could reflect reduced hormone receptor levels in rats fed the low fat diet, which may be marginally deficient in essential fatty acids. In fact, no differences are

observed in prolactin receptor levels of DMBA- or NMU-induced mammary tumors in rats fed a 20.0% high fat diet or a 4.5% control fat diet (62,63). Ip and Ip (64) reported that estrogen receptor levels in mammary tumors were the same in rats fed moderate (5.0%) and high (20.0%) levels of dietary fat but were reduced in rats fed a low (0.5%) fat diet. Also, progesterone receptor levels were unaffected by dietary fat. Thus, while dietary fat consumption may subtly influence endocrine secretion and/or the responsiveness of incipient mammary tumor tissue to mammotrophic hormones, the processes do not appear to be influenced sufficiently to affect the genesis and/or growth of murine mammary tumors (12,13).

The immune system plays an integral role in the tumorigenic process (65) and has been implicated in mediating the effects of high dietary fat in mammary tumorigenesis. Diets high in polyunsaturated fats suppress immune function (66). Diets high in polyunsaturated fatty acids have been reported to prolong skin allografts in rodents (67), to enhance immunosuppressive therapy following renal transplantation (68), to decrease macrophage activity (69) and to reduce peripheral lymphocyte concentrations (70). However, other reports show no effects on immune activity (71-74). Thus, while mediation of the promotion of mammary tumorigenesis by high levels of dietary fat through immunosuppression is an attractive concept, the complexities of this system and the inconsistencies of the data prevent an adequate assessment of the hypothesis.

Prostaglandins also have been implicated in the promotion of mammary tumorigenesis by dietary fat. Since linoleic and linolenic acids are precursors in the biosynthesis of certain prostaglandins, diets with elevated levels of these fatty acids may provide the substrate for increased synthesis of those prostaglandins. Dupont et al. (75) reported a positive relationship between dietary levels of linoleate and prostaglandin biosynthesis. Also, indomethacin, an inhibitor of prostaglandin biosynthesis (76), can block the stimulatory effects of high dietary fat on mammary tumorigenesis (77,78).

Because certain unsaturated fatty acids stimulate, whereas saturated fatty acids inhibit, the growth of cell cultures of normal and neoplastic rat mammary glands (39-41), dietary lipids may directly stimulate mammary tumor development and growth. The lipid composition of mammary tumors reflects qualitatively and quantitatively the fatty acid content of the diet (78-80). Changes in fatty acid composition of cell membranes may influence cellular biophysical phenomenon, including membrane fluidity, macromolecular mobility, receptor availability, enzyme activation, cyclic nucleotide and prostaglandin biosynthesis and amino acid and carbohydrate transport processes. These changes may provide conditions favorable to inducing cell division or may interfere with the processes that control cell division. Thus, the mitogenic signal that ultimately results in increased mammary tumor development and growth may be the alteration of the lipid composition of the cellular membrane itself.

INTERCELLULAR COMMUNICATION AND TUMOR PROMOTION

The development of many experimental tumors following application of a carcinogen (initiator) at subthreshold levels depends upon subsequent treatment with a "noncarcinogenic" tumor promoter. Classically, when DMBA or another carcinogen is applied to mouse skin at subthreshold doses, a high incidence of local tumors is observed only in animals subsequently treated with a tumor promoter that is noncarcinogenic when applied alone (81,82). This observation has been expanded and adapted to include tumor promotion in other tissues. For example, in mammary carcinogenesis, development of tumors following DMBA or NMU treatment is dependent upon subsequent exposure to mammotrophic hormones (83,84). Removal of pituitary or ovarian influences results in nearly complete suppression of mammary tumor development (85,86). An understanding of how the growth of these initiated, potentially tumorigenic cells is suppressed or, conversely, how tumor promoters are able to reverse this suppression and allow for development of the tumor, would contribute to understanding the tumorigenic process.

One theory to explain suppression of the proliferation of initiated, latent tumor cells is that normal cells surrounding the transformed or initiated foci exert a growth inhibitory influence. Indeed, growth of transformed C3H10T1/2 cells is inhibited when they are cocultured with nontumorigenic "normal" cells in vitro (87,88). This effect is overcome when tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate (TPA) is added to the culture medium (89,90).

Intercellular communication is thought to be involved in a wide variety of developmental processes and in control of cellular growth and differentiation (91-93). Recently it was suggested that intercellular communication may also have a role in tumor promotion (94). A specific type of intercellular communication, metabolic cooperation, involves the passage of low molecular weight and possibly growth-regulating molecules through membrane structures called gap junctions. In 1979, Yotti et al. (95) and Murray and Fitzgerald (96) independently reported that metabolic cooperation between cells in culture is blocked by the classical tumor promoter TPA. Since these initial reports, many other tumor-promoting compounds have been shown to inhibit metabolic cooperation (94,97-107). Table 2 lists compounds that inhibit gap junction-mediated intercellular communication. A diverse range is listed, including naturally occurring compounds, environmental toxicants and/or pollutants, drugs, food additives and nutritionally related compounds. Thus, inhibition of intercellular communication by tumor promoters is not limited to related compounds, nor is it specific to a certain class of compounds. Also, correlations linking the efficacy of these compounds as tumor promoters to their ability to block metabolic cooperation in vitro have been described (94,107). Accordingly, a hypothesis has evolved whereby the enhanced proliferation of "initiated" cells and subsequent tumor development induced by tumor promoters (e.g., TPA) is caused by inhibiting intercellular communication, resulting in a blockade of the transfer of growth inhibitory signals via gap junctions. Increased proliferation of initiated cells in the presence of tumor promoters allows these cells to gain a selective growth advantage and increases the probability for further mutational events, resulting in autonomous, promoter-independent growth (i.e., tumor progression).

TABLE 2

Tumor Promoters That Inhibit Intercellular Communication[a]

	Tumor-promoted tissue
Naturally occurring compounds	
TPA (and other tumor-promoting phorbol esters)	Skin
Teleocidin	Skin
Bile acids (deoxycholic, lithocholic acids)	Colon
T-2 toxin	Esophagus
Aplasiatoxin	Skin
Environmental toxicants and/or pollutants	
2,4,5,2',4',5' Hexabromobiphenyl	Liver
2,4,5,2',4',5' Hexachlorobiphenyl	Liver
Benzoyl peroxide	Skin
Anthralin	Skin
Di-(2-ethyrexyl)-phthalate	Liver
Chlordane	Liver
Cigarette smoke condensates	Skin
Lindane	Liver
2,4-Dinitrofluorobenzene	Skin
DDT-(1.1.1-trichloro-2,2-bis p-chlorophenyl ethane)	Liver
Kepone	Liver
Aldrin	Liver
Dieldrin	Liver
Mirex	Liver
NTA-(trisodium nitrilotriacetate monohydrate)	Kidney
Drugs, food additives	
Phenobarbitol	Liver
Chlorpromazine	Liver
BHT (butylated hydroxytoluene)	Liver
Nutritionally related compounds	
Saccharin	Urinary bladder
Cyclamates	Urinary bladder
Unsaturated fatty acids	Mammary gland, others
Retinoic acid (high concentrations)	Skin

[a]Taken in part from ref. 94.

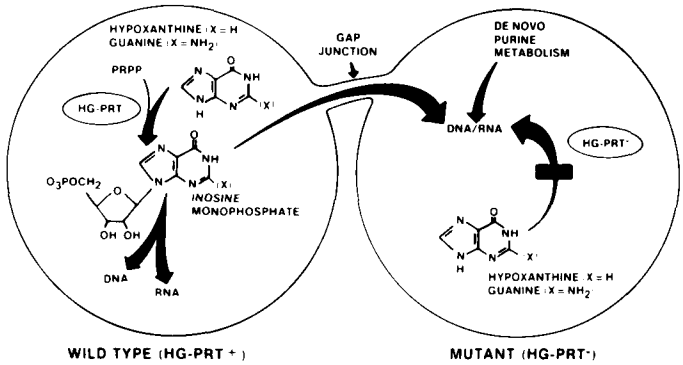

FIG. 1. Diagram illustrating the principle of the V79 Chinese hamster cell metabolic cooperation assay to measure gap junction-mediated intercellular communication (ref. 94).

IN VITRO METABOLIC COOPERATION ASSAY SYSTEM

An in vitro assay system to examine the influence of various environmental compounds on intercellular communication and to study the mechanisms of tumor promotion was developed by Trosko and colleagues (108,109). Gaudin et al. (110) observed that TPA enhanced the recovery of ultraviolet (UV) light-induced 6-thioguanine (hypoxanthine guanine phosphoribosyl-transferase-deficient [HG-PRT$^-$])-resistant Chinese hamster cells in culture. It was subsequently shown by Yotti et al. (95) that the enhancement of recovery of 6-thioguanine (6-TG)-resistant mutant cells was due to a blockade of metabolic cooperation by TPA.

Metabolic cooperation, on which our in vitro assay is based, is the phenomenon in which low molecular weight and possibly growth-regulating molecules are passed from the cytoplasm of one cell to an adjacent cell via membrane structures called gap junctions. The phenomenon is schematically illustrated in Figure 1. HG-PRT is an enzyme involved in the purine salvage pathway. Normal wild-type V79 Chinese hamster cells that contain HG-PRT (HG-PRT$^+$), grown in vitro in culture medium containing 6-TG, take up 6-TG, phosphoribosylate it to a lethal metabolite (6-thioguanosine monophosphate), are unable to proliferate and die. However, V79 Chinese hamster cells that have been mutated by x-irradiation or UV radiation and lack HG-PRT (HG-PRT$^-$) are unable to metabolize 6-TG and therefore continue to proliferate in medium containing 6-TG. (Because HG-PRT is a nonessential enzyme, metabolism of these cells is otherwise normal.) When wild-type (HG-PRT$^+$) V79 cells are cocultivated with mutant (HG-PRT$^-$) V79 cells in medium containing 6-TG, the HG-PRT$^+$ cells take up the 6-TG, phosphoribosylate it and transfer the lethal metabolite (6-TG monophosphate) via gap junctions to the mutant HG-PRT$^-$ cells if they are in physical contact. Transfer of 6-TG monophosphate is dependent upon the presence and proper functioning of gap junctions. Sufficient transfer of 6-TG monophosphate will kill the HG-PRT$^-$ cells. Therefore, recovery of the mutant HG-PRT$^-$ V79 cells is inversely related to the amount of metabolic cooperation between HG-PRT$^+$ 6-TG-sensitive (6-TG^S) and HG-PRT$^-$ 6-TG-resistant (6-TG^R) cells (i.e., increased recovery of HG-PRT$^-$ 6-TG^R cells indicates decreased metabolic cooperation). Addition to the culture medium of chemicals that decrease metabolic cooperation will result in increased recovery of 6-TG^R cells. As stated previously, a number of known tumor promoters inhibit the transfer of 6-TG monophosphate from 6-TG^S to 6-TG^R V79 Chinese hamster cells and increase the recovery of HG-PRT$^-$ cells (i.e., inhibit metabolic cooperation).

Figure 2 summarizes the experimental protocol of the V79 metabolic cooperation assay system. Details of the procedure have been published elsewhere (108,109); they are summarized as follows: Cytotoxicity is initially tested by examining the effect of various fatty acid doses on the colony-forming ability of 100 V79 Chinese hamster cells in 6-cm tissue culture dishes. All fatty acids are dissolved and diluted in 100% ethanol and are added to the cultures about 3 hr after the cells are seeded. Dilutions are made such that the final concentration of ethanol in the culture medium is less than 0.5%. Cultures are incubated in humidified air with 5% CO_2 at 37 C until colonies have grown big enough to be scored visually (usually 7-9 days), with medium changes after three and six days. The culture medium is a modified Eagle's minimal essential

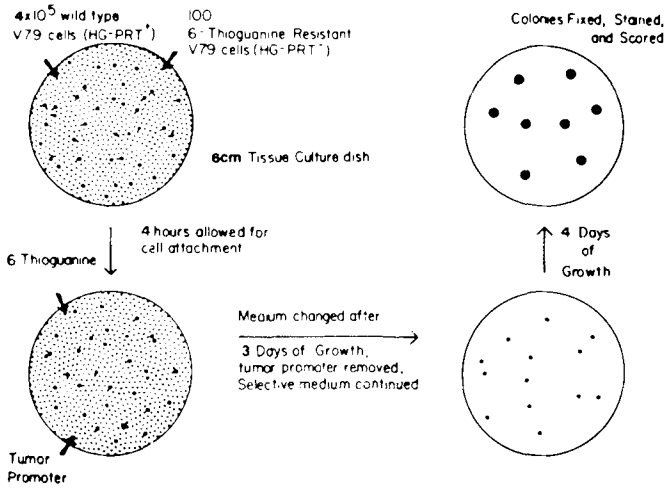

FIG. 2. Diagram describing the experimental protocol of V79 cell metabolic cooperation assay (ref. 94).

medium with Earle's salts, supplemented with 100% increase of all nonessential amino acids, 50% increase in all vitamins and essential amino acids except glutamine, 1.0 mM sodium pyruvate, antibiotics (100 IU penicillin and 100 IU streptomycin per ml medium) and 3% fetal bovine serum. Colonies are rinsed with 0.85% saline, fixed and stained with crystal violet and scored visually.

Once appropriate noncytotoxic dose ranges are established, the effect of various fatty acids on metabolic cooperation is assessed. Wild-type HG-PRT$^+$ 6-TG^S cells are seeded in 6-cm dishes (4×10^5 cells per plate) with 100 HG-PRT$^-$ 6-TG^R cells in 5 ml of culture medium. After 3–4 hr, various doses of the test fatty acids are added. Ethanol is added (final concentration of 0.5%) to a series of plates as a solvent control. TPA (1–2 ng/ml) is added to another series of plates as a positive control. After test chemicals have been added, 6-TG (10 μg/ml) is added to all plates. Cells are cultured for three days, after which the culture medium is changed and replaced with selective culture medium containing only 6-TG (10 μg/ml). Culture medium is changed once again on day 6. Cytotoxicity is confirmed by testing the effect of the same concentrations of fatty acids on the colony-forming ability of 100 6-TG^R metabolic cooperation-deficient (MC$^-$) mutants (111) cocultured with 4×10^5 6-TG^S cells in 6-cm tissue culture dishes in the presence of 6-TG (10 μg/ml). This method allows for more accurate assessment of cytotoxicity since, unlike the previous cytotoxicity evaluation (i.e., 100 6-TG^R cells cultured alone), the cell density conditions are identical to those used in the metabolic cooperation determinations.

FATTY ACID EFFECT ON METABOLIC COOPERATION

In view of the promotion of mammary tumorigenesis by high fat diets and the possible role of inhibition of gap junction-mediated intercellular communication in tumor promotion, a series of experiments was conducted to assess the influence of fatty acids on metabolic cooperation (14,112). It is apparent from the data presented in Figures 3–5 that unsaturated fatty acids inhibit metabolic cooperation at noncytotoxic concentrations, whereas

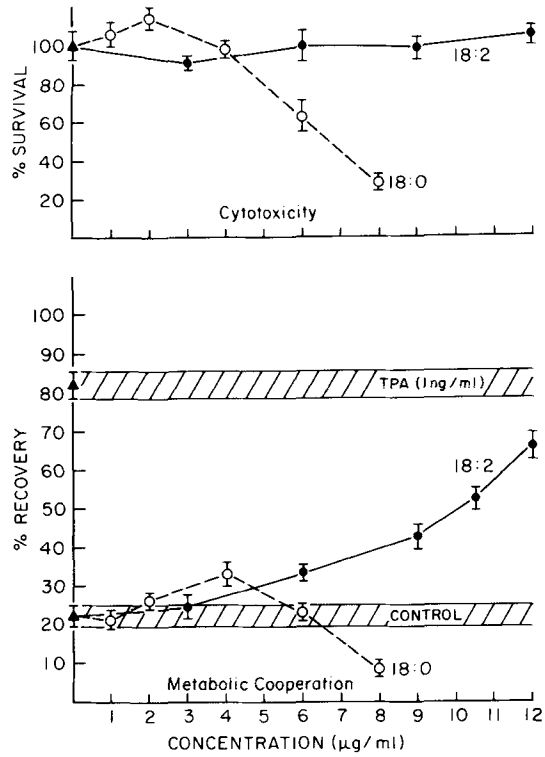

FIG. 3. Effect of linoleic acid (18:2) and stearic acid (18:0) on cytotoxicity (% survival) and on metabolic cooperation (% recovery) between Chinese hamster V79 cells. Negative (solvent) and positive (TPA-treated) controls shown in shaded areas with SEM (Ref. 112).

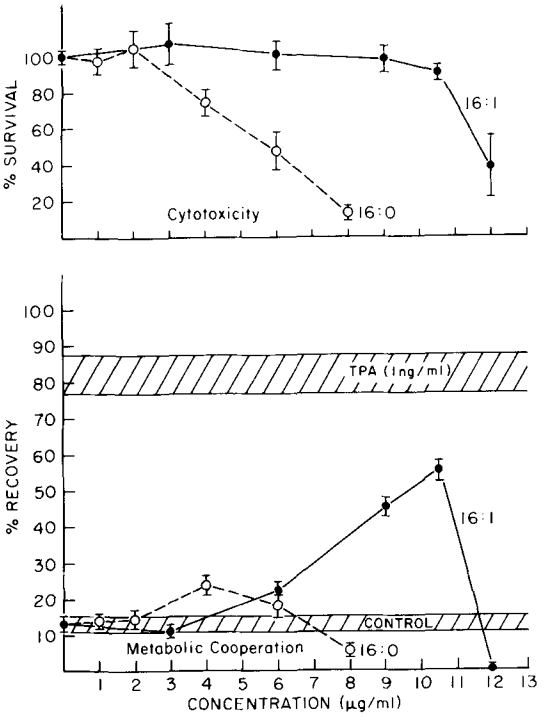

FIG. 4. Effect of palmitoleic acid (16:1) and palmitic acid (16:0) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

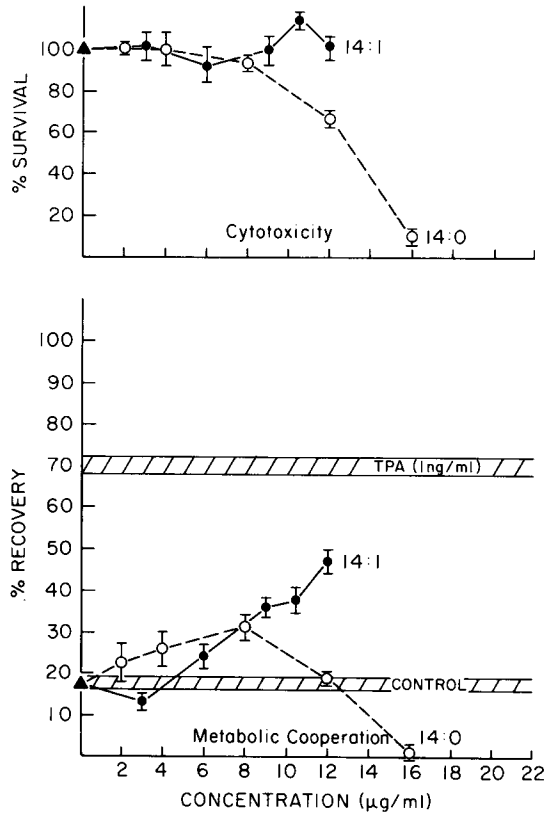

FIG. 5. Effect of myristoleic acid (14:1) and myristic acid (14:0) on cytotoxicity (% survival) and on metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

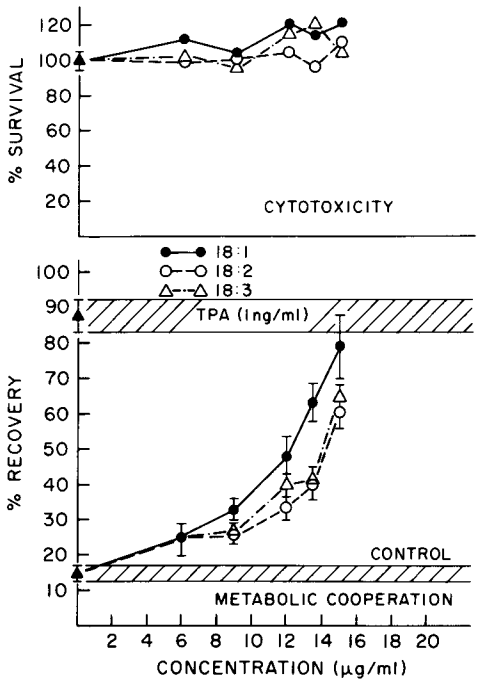

FIG. 6. Importance of the degree of unsaturation on the inhibition of metabolic cooperation by unsaturated fatty acids. Effects of oleic (18:1), linoleic (18:2) and linolenic acids (18:3) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

saturated fatty acids fail to do so at any concentration. The unsaturated fatty acids—linoleic (18:2; Fig. 3), palmitoleic (16:1; Fig. 4) and myristoleic (14:1; Fig. 5)—significantly increase the recovery of 6-TG^R cells cocultured with 6-TG^S cells in a concentration-dependent manner at noncytotoxic concentrations. However, the saturated fatty acids—stearic (18:0; Fig. 3), palmitic (16:0; Fig. 4) and myristic (14:0; Fig. 5)—failed to significantly influence the recovery of 6-TG^R cells at noncytotoxic concentrations. Other unsaturated 18-carbon fatty acids, linolenic (18:3) and oleic (18:1), also inhibit metabolic cooperation, resulting in increased recovery of 6-TG^R cells (Fig. 6).

To assess the importance of the degree of unsaturation in inhibiting metabolic cooperation by unsaturated fatty acids, the abilities of 18:1, 18:2 and 18:3 to increase 6-TG^R cell recovery were evaluated. Figure 6 shows that no relationship exists between degree of unsaturation and inhibition of metabolic cooperation. Oleic acid (18:1) appears slightly more efficacious than linoleic (18:2) or linolenic (18:3) acids, which are about equal, in inhibiting metabolic cooperation.

There is an apparent association between the ability of unsaturated fatty acids to inhibit metabolic cooperation and the carbon chain length. When fatty acids with the same degree of unsaturation (i.e., one double bond) but different chain lengths are compared, those with longer chain lengths inhibit metabolic cooperation more than those with shorter chain lengths. Figure 7 shows that

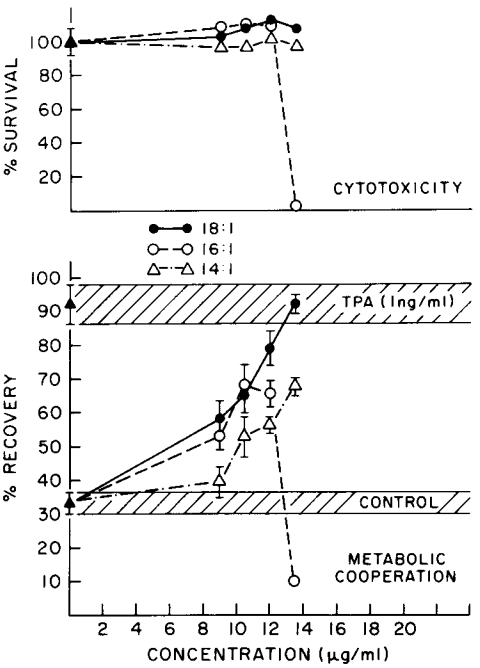

FIG. 7. Influence of carbon chain length on the inhibition of metabolic cooperation by unsaturated fatty acids. Effects of oleic (18:1), palmitoleic (16:1) and myristoleic acids (14:1) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

oleic acid (18:1) inhibits metabolic cooperation more than palmitoleic (16:1) or myristoleic (14:1) acids. Palmitoleic acid (16:1) appears to inhibit metabolic cooperation slightly more than myristoleic acid (14:1).

Geometric isomerism also appears important in determining the effect of unsaturated fatty acids on metabolic cooperation. Fatty acids with the *cis*-double bond orientation are more efficacious than the corresponding *trans*-oriented fatty acids. *Cis*-oleic acid (*cis* 18:1) is much more effective than elaidic acid (*trans* 18:1) (Fig. 8), and *cis*-palmitoleic acid (*cis* 16:1) is more effective than palmitelaidic acid (*trans* 16:1) (Fig. 9). However, the differences between *cis* and *trans* 16:1 are much less dramatic than those between *cis* and *trans* 18:1. These data thus suggest that the *cis* double bond orientation appears important in the inhibition of metabolic cooperation by certain unsaturated fatty acids, but less important for other fatty acids.

To further examine the influence of fatty acids on intercellular communication, the effect of medium (11-carbon) and short (6-carbon) chain fatty acids on metabolic cooperation was assessed. Both undecanoic (11:0) and undecylenic (11:1) acids significantly inhibit metabolic cooperation (Fig. 10). However, compared with oleic acid (18:1), the inhibition of metabolic cooperation by 11:0 and 11:1 is apparent only at much higher concentrations. Hexanoic (6:0) and sorbic (6:2) acids do not influence metabolic cooperation at concentrations up to 100 μg/ml (Fig. 11) or 200 μg/ml (data not shown). Thus, it appears that the importance of unsaturation and the ability of fatty acids to inhibit metabolic cooperation is reduced in medium and short chain fatty acids.

CONCLUSIONS

High levels of dietary fat clearly promote many types of experimental cancers and are implicated in the etiology of some human cancers. Many mechanisms, including endocrine-related and immune-mediated ones, have been proposed to explain the promoting influence of high polyunsaturated fat diets on mammary tumorigenesis. A review of available information indicates that a direct influence of dietary fat promoting growth of incipient mammary tumor tissue is the most likely interpretation. One way in which unsaturated fatty acids may directly influence the growth regulation of mammary tumor tissue, and thereby promote tumor development, is by inhibiting gap junction-mediated intercellular communication. This inhibition is associated with many known tumor promoters. Indeed, unsaturated fatty acids such as oleic, linoleic, linolenic, palmitoleic and myristoleic inhibit metabolic cooperation, a process that depends on gap junction-mediated intercellular communication. While there is no apparent relationship between the degree of unsaturation of fatty acids and their ability to inhibit metabolic cooperation, long chain unsaturated fatty acids (i.e., C18) are more effective than medium (C11) or short (C6) chain ones. The *cis* double bond orientation is important for certain fatty acids to inhibit metabolic cooperation. Also, unsaturation is less important in medium and short chain fatty acids than in longer (≥C14) chain ones.

The mechanism by which unsaturated fatty acids inhibit metabolic cooperation is unclear. Incorporation of unsaturated fatty acids into cell membranes may alter

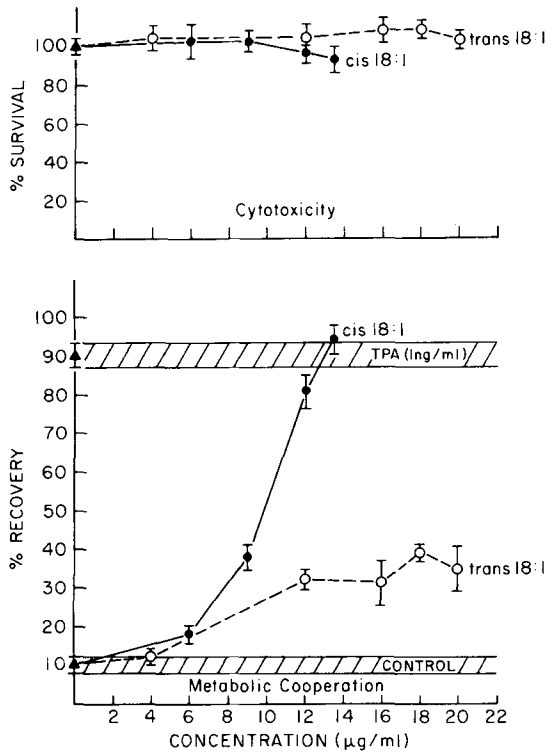

FIG. 8. Importance of *cis*-double bond orientation on the inhibition of metabolic cooperation by unsaturated fatty acids. Effects of *cis*-oleic acid (*cis* 18:1) and elaidic acid (*trans* 18:1) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

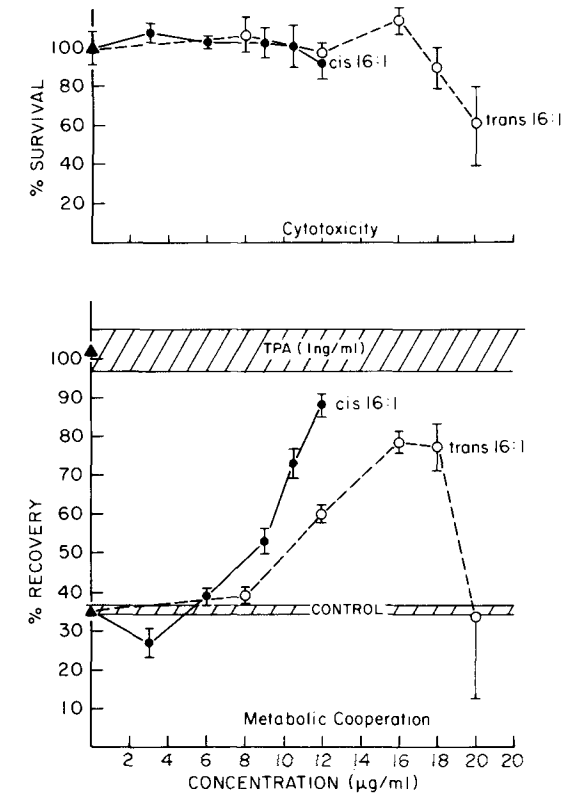

FIG. 9. Importance of *cis*-double bond orientation on the inhibition of metabolic cooperation by unsaturated fatty acids. Effects of *cis*-palmitoleic acid (*cis* 16:1) and palmitelaidic acid (*trans* 16:1) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells (ref. 112).

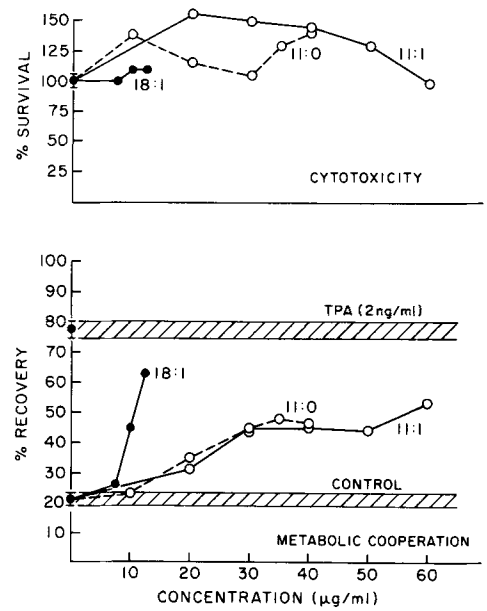

FIG. 10. Effect of undecanoic acid (11.0) and undecylenic acid (11:1) on cytotoxicity (% recovery) and metabolic cooperation (% recovery) between Chinese hamster V79 cells.

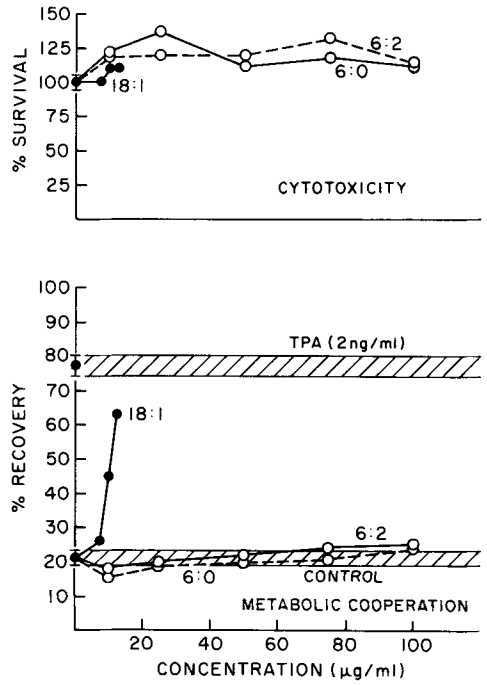

FIG. 11. Effect of hexanoic acid (6:0) and sorbic acid (6:2) on cytotoxicity (% survival) and metabolic cooperation (% recovery) between Chinese hamster V79 cells.

many biophysical properties of the membrane, including membrane fluidity, receptor and macromolecular availability and transport mechanisms. Since gap junctions are membrane structures, changes in the membrane microenvironment could alter their functional capacity. Incorporation of unsaturated fatty acids into the membrane increases fluidity (80), which could result in a destabilization of gap junction structure and thereby cause a decrease in gap junction-mediated intercellular communication (i.e., metabolic cooperation). However, since no greater inhibitory effects were observed with more unsaturated fatty acids (i.e., 18:1, 18:2, 18:3), such mechanisms involving membrane fluidity are unlikely.

Unsaturated lipid may also inhibit intercellular communication by altering enzyme activity, in particular, the Ca^{2+}-activated, phospholipid-dependent, diacylglycerol-sensitive protein kinase C. Protein kinase C is a widely distributed cyclic AMP-independent protein kinase (or group of kinases) recently implicated in mediating many cellular processes, including tumor promotion, in normal and neoplastic tissues (113,114). Its activity is increased by tumor promoters that inhibit intercellular communication (such as TPA) and by diacylglycerol compounds that contain unsaturated fatty acids (113,115-118). Recently, Gainer and Murray (119) and Aylsworth et al. (112) reported that unsaturated diacylglycerol compounds inhibit metabolic cooperation, suggesting a role for protein kinase C in the control of intercellular communication.

Linoleic and linolenic acids may influence the biosynthesis of certain prostaglandins and thereby affect a variety of cellular processes, including intercellular communication (120). However, the inhibition of metabolic cooperation by other unsaturated fatty acids thought to be not involved in prostaglandin biosynthesis (oleic, palmitoleic, and myristoleic acids) suggests that altered prostaglandin biosynthesis is not involved in mediating these effects.

Finally, lipid peroxidation products may also have a role in mediating the inhibitory effects of unsaturated fatty acids on metabolic cooperation. Oxygen free radicals generated by peroxidation of unsaturated fatty acids may cause dysfunction of gap junction structures, thereby reducing gap junction-mediated intercellular communication. However, mechanisms involving lipid peroxidation also are unlikely, because no increased inhibition of metabolic cooperation was observed with fatty acids containing increasing degrees of unsaturation.

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