Dietary Butter Protects against Ultraviolet Radiation-Induced Suppression of Contact Hypersensitivity in Skh:HR-1 Hairless Mice[1,2]

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ABSTRACT Dietary fats modulate a wide variety of T cell functions in mice and humans. This study examined the effects of four different dietary fats, predominantly polyunsaturated sunflower oil, margarine, and predominantly saturated butter, clarified butter, on the T cell-mediated, systemic suppression of contact hypersensitivity by ultraviolet radiation in the Skh:HR-1 hairless mouse. Diets containing either 200 g/kg or 50 g/kg butter or clarified butter as the sole fat source protected against systemic photoimmunosuppression, whether the radiation source was unfiltered ultraviolet B (280–320 nm) or filtered solar simulated ultraviolet radiation (290–400 nm), in comparison with diets containing either 200 or 50 g/kg margarine or sunflower oil. There was a linear relationship ($r > 0.9$) between protection against photoimmunosuppression and the proportion of clarified butter in mice fed a series of 200 g/kg mixed fat diets that provided varying proportions of clarified butter and sunflower oil. The dietary fats did not modulate the contact hypersensitivity reaction in unirradiated animals. The observed phenomena were not primarily due to the carotene, tocopherol, cholecalciferol, retinol, lipid hydroperoxide or the nonfat solid content of the dietary fats used and appeared to be a result of the different fatty acid composition of the fats. J. Nutr. 126: 681–692, 1996.

INDEXING KEY WORDS:

• *ultraviolet rays* • *contact hypersensitivity*
• *dietary fat* • *immunosuppression* • *hairless mouse*

The suppressive effect of ultraviolet radiation on contact and delayed type hypersensitivity reactions has been extensively studied in animals and humans. It was demonstrated to be due to an active process that results in a state characterized by T lymphocyte mediated antigen-specific unresponsiveness (Noonan and De Fabo 1993). Dietary fats were demonstrated to modulate many T cell-mediated events and therefore may potentially alter the immunological consequences of ultraviolet radiation exposure (Erickson 1986, Mertin and Mertin 1988).

The aim of this study was to examine the effects of four fats relevant to human nutrition: sunflower oil, margarine (a blended derivative of sunflower oil), butter and clarified butter (butter with the nonfat solids removed) on ultraviolet radiation-induced immunosuppression using the systemic contact hypersensitivity reaction to monitor T cell-mediated function. The diets used contained 50 or 200 g/kg fat (5% fat or 20% fat, respectively), representing ~11.6 and 39.7% of energy intake as fat, respectively. The high fat diet approximates the average North American's fat intake, which has been estimated to be ~35% of energy intake (Meydani et al. 1993). Because the diets used in this study do not represent the variety of fats consumed by the average human, a series of relevant mixed fat diets also was examined.

MATERIALS AND METHODS

Animals. Inbred female albino Skh:HR-1 mice 8–15 wk old produced by a conventional colony maintained in this department were used. Groups of mice were housed in wire top plastic cages on vermiculite bedding

[1] Supported by a grant from the Dairy Research and Development Corporation of Australia, Glen Iris, VIC.

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Manuscript received 2 February 1995. Initial review completed 13 April 1995. Revision accepted 6 November 1995.

(Boral, Camellia, Australia) under ambient yellow light provided by GEC F40GO fluorescent tubes, with a 12-h light/dark cycle and at a temperature of 25°C. Before beginning the experiments, mice had free access to a pelleted complete nonpurified rodent diet (Doust and Rabbidge, Sydney, Australia) and water. Age-matched groups of 10 mice (5 irradiated and 5 unirradiated controls) were used for each treatment group. The production and maintenance of the experimental mice conformed to the Australian National Health and Medical Research Council guidelines for the care and use of laboratory animals.

Semi-purified diets. The fats were either sunflower oil (without added antioxidant or colors), table margarine (Vegetable Oils, Sydney, Australia; a blend of approximately two-thirds sunflower oil and one-third partially hydrogenated cottonseed oil), butter (Allowrie Foods Australia, Sydney, Australia) and clarified butter. Mixed 20% fat diets that contained varying proportions of clarified butter and sunflower oil were also used. Butter was clarified by melting at 50°C, decanting the liquefied fat and discarding the sediment that contained the nonfat solids. The efficacy of the clarification process was examined using the modified Kohman method (Bianco et al. 1978). Analyses of triplicate samples of butter yielded 3.26 ± 0.20 g of nonfat solids per 100 g. After clarification, the nonfat solid concentration was reduced to 0.10 ± 0.05 g per 100 g. All fats were stored at 2°C in the dark. Sunflower oil was supplied in 20-l batches and was stored under N_2 gas. The margarine and butter were purchased from a retail outlet and were stored for ≤10 d.

The protein source used was soybean protein isolate (Supro 500E, Protein Technologies, St. Louis, MO), the carbohydrate source used was sucrose, the fiber was finely ground wheat straw and the vitamins and minerals used were according to recommended daily requirements for mice (AIN-76®, American Institute of Nutrition 1977; **Table 1**). The diets were mixed in a domestic food mixer and stored at 2°C for up to 10 d.

The diets were fed simultaneously to groups of mice in weighed daily aliquots at the same time of day and in an isocaloric manner so that the daily ration of each diet contained approximately 79 kJ of energy per mouse. The diets were prefed for 2 or 4 wk before the beginning of irradiation or sampling for epidermal fatty acid composition, as described below, and were then fed for the entire duration of all experiments. The content of all components of the daily ration, except the fat and the carbohydrate, were identical on a per unit energy basis (Table 1), with the carbohydrate concentration altered to compensate for the different levels of fat energy.

Analysis of nonvolatile fatty acid composition of the fats. Samples of the fats were stored in glass containers with Teflon-lined lids in an N_2 environment at −145°C. Using heptadecanoic acid (Sigma-Aldrich, Castle Hill, Australia) as the internal standard, lipid extraction was by the chloroform-methanol extraction method of Folch et al. (1957), followed by saponification and methyl esterification using the acid, methanol and ammonium chloride method of Hartman and Lago (1973). Gas-liquid chromatography was performed by using a 3.05-m long, 4-mm i.d. glass column with 10% Silar 10C as the liquid phase (Alltech Australia, North Strathfield, Australia) and high purity N_2 as the carrier. Fatty acids were identified using the retention times of various purified fatty acid methyl-ester standards (Alltech Australia).

TABLE 1

Composition of semipurified diets

Dietary component	5% fat diet	5% fat diet: intake per daily ration	20% fat diet	20% fat diet: intake per daily ration
	g/kg	*g*	*g/kg*	*g*
Soybean protein	201.4	0.95	236.6	0.95
Sugar[1]	639.2	3	434.9	1.74
Fat	50.0	0.235	200.0	0.80
DL-methionine	3.0	0.014	3.5	0.014
Choline chloride	0.8	0.004	0.9	0.004
Vitamin mixture[2]	10.0	0.05	11.9	0.05
Mineral mixture[2]	31.0	0.15	36.4	0.15
Finely ground hay	64.6	0.30	75.9	0.30

[1] Equality of energy intake from carbohydrate (sugar) plus fat (5 or 20%) was maintained by varying the sugar concentration.
[2] As per AIN-76™ (American Institute of Nutrition 1977).

The fatty acid composition of butter and margarine purchased at different times did not differ greatly (average content of each fatty acid varied <5%) during the course of the experiments. There was no detectable alteration in fatty acid composition with storage under the conditions described. The nonvolatile fatty acid composition of the fats is displayed in **Table 2**. Clarification of the butter did not greatly alter its nonvolatile fatty acid composition. There were large differences in the levels of 18:1, 18:2 and the ratio of unsaturated:saturated fatty acids in the different fats. 16:1, 12:0 and shorter chain fatty acids were detected only in butter and clarified butter. 18:3 was detected only in the margarine. 20:2 was detected only in the sunflower oil. The margarine contained 9.5 g per 100 g of *trans*-18:1.

Analysis of nonvolatile fatty acid composition of the epidermis. Groups of three mice were fed the different diets for 2 or 4 wk and were killed by cervical dislocation. The dorsal skins were excised, immediately chilled and stored in glass containers with Teflon-lined lids at −145°C in an N_2 environment. The epidermises were collected by heat shock (Reeve et al. 1993), and the epidermal material was then homogenized for 5 min in cold chloroform:methanol 2:1 (v/v) in an N_2

TABLE 2

Nonvolatile fatty acid composition of dietary fats[1]

Fatty acid	Sunflower oil	Margarine	Butter	Clarified butter
	mol/100 mol			
6:0	ND[2]	ND	0.91 ± 0.02	0.94 ± 0.01
8:0	ND	ND	1.34 ± 0.01	1.33 ± 0.01
10:0	ND	ND	3.40 ± 0.01	3.35 ± 0.01
12:0	ND	ND	4.20 ± 0.01	4.16 ± 0.01
14:0	0.14 ± 0.02	0.33 ± 0.01	14.82 ± 0.04	14.73 ± 0.02
16:0	8.21 ± 0.04	12.8 ± 0.05	40.89 ± 0.03	40.68 ± 0.05
16:1	ND	ND	3.77 ± 0.01	3.80 ± 0.01
18:0	3.81 ± 0.01	4.70 ± 0.01	9.07 ± 0.03	9.14 ± 0.04
18:1	27.51 ± 0.03	39.44 ± 0.62	18.90 ± 0.05	19.02 ± 0.06
18:2	59.73 ± 0.09	40.77 ± 0.05	1.37 ± 0.01	1.47 ± 0.04
18:3	ND	3.07 ± 0.07	1.35 ± 0.01	1.39 ± 0.02
20:2	0.61 ± 0.01	ND	ND	ND
U:S ratio[3]	7.17	4.67	0.34	0.35

[1] Results are means ± SEM of three separate analyses and are expressed as mol of fatty acid/100 mol of total fatty acids.
[2] ND = not detected.
[3] Ratio of unsaturated to saturated fatty acids.

environment. The extraction, saponification and methyl esterification and analysis were then identical to that previously described for the dietary fats.

Analysis of the antioxidants in the fats. Tocopherols, carotenes and lipid hydroperoxides were analyzed by the HPLC postcolumn chemiluminescence (HPLC-PCCL)[4] method of Sattler et al. (1994). Briefly, 10-mg samples of the different fats were dissolved in 10 ml isopropanol and vortexed for 1 min, and the insoluble material was removed by filtration through a 0.2-μm nylon Acrodisc 13 filter (Gelman, Sydney, Australia). Aliquots (20 μl) of each solubilized fat were then analyzed by HPLC-PCCL using appropriate standards.

The α-tocopherol concentration of triplicate samples of the fats was as follows: butter 3.5 ± 0.2 (mean ± SEM) mg/100 g of fat; clarified butter 4.6 ± 0.1; margarine 21.1 ± 0.2; sunflower oil 50.4 ± 0.8. The γ-tocopherol concentration of typical samples of the fats was as follows: butter 0; clarified butter 0; margarine 20.6 ± 0.3 (mean ± SEM) mg/100 g; sunflower oil 4.2 ± 0.4. The β-carotene content of triplicate samples of the fats was as follows: butter 6.8 ± 0.4 (mean ± SEM) mg/100 g of fat; clarified butter 7.9 ± 0.3; margarine 6.6 ± 0.6; sunflower oil 0. Lipid hydroperoxides were not present in the fats.

Analysis of retinol and cholecalciferol concentration of the fats. The concentrations of retinol and cholecalciferol in the various dietary fats were determined at the Australian Government Analytical Laboratories by HPLC after hydrolysis and extraction. The analyses were performed to National Association of Testing Authorities, Australia (NATA) accreditation and Australian Quality Assurance (AQA) standards. The retinol concentration of the fats was as follows: butter 1500 μg/100 g of fat; clarified butter 1200; margarine 780; sunflower oil < 5. The per gram and per unit energy total vitamin A levels of the diets were calculated from the retinol and β-carotene analyses and are displayed in **Table 3**. The various dietary fats all contained similar low levels of cholecalciferol (<50 μg/100 g of fat).

Irradiation of mice. The ultraviolet fluorescent tube light sources, their spectral outputs and irradiation techniques were described previously (Reeve et al. 1993). Five mice from each group of 10 (the remaining animals acting as unirradiated controls) received one minimal edematous dose of unfiltered ultraviolet B (UVB, 280–320 nm) on three consecutive days; the total cumulative dose was 1.78 J/cm^2 UVB and 0.27 J/cm^2 ultraviolet A (UVA, 320–400 nm). Alternatively, five mice per group of 10 received 4 wk (5 consecutive days per wk) of incremental simulated solar ultraviolet radiation (290–400 nm); the initial exposure was 0.75 of the minimal edematous dose and was incremented weekly by 20% of the initial dose to overcome acquired tolerance and maintain the biological response (Reeve et al. 1993). The cumulative exposure was 10.33 J/cm^2 UVB and 51.53 J/cm^2 UVA.

Induction of the contact hypersensitivity reaction. On the 7th and 8th d after the first exposure to UVB or the last exposure to solar simulated ultraviolet radiation, mice were sensitized to 2,4-dinitrofluorobenzene (Sigma-Aldrich) by painting 50 μl of a freshly prepared 0.3% solution (v/v) in analytical grade acetone over the largest possible area of ventral

[4] Abbreviations used: AQA, Australian Quality Assurance; HPLC, high-performance liquid chromatography; HPLC-PCCL, high-performance liquid chromatography-postcolumn chemiluminescence; NATA, National Association of Testing Authorities, Australia; UVA, ultraviolet A; UVB, ultraviolet B.

TABLE 3

Total vitamin A levels of diets

Diet	Total vitamin A concentration	
	Retinol equivalents/g[1]	*Retinol equivalents per daily ration*
20% Sunflower oil	4	16
20% Margarine	31	124
20% Butter	36	146
20% Clarified butter	38	153
5% Sunflower oil	3	15
5% Margarine	10	47
5% Butter	11	54
5% Clarified butter	11	56

[1] Retinol equivalent of Vitamin A = 0.3 μg of retinol = 0.6 μg of β-carotene.

(unirradiated) skin at the same time of day. One week after the first sensitization, mice were challenged by painting 5 μl of a freshly prepared 0.2% solution (v/v) of 2,4-dinitrofluorobenzene on both surfaces of both pinnae. The thicknesses of both pinnae were measured before and 20 h after challenge by using a spring micrometer (Mercer, St. Albans, United Kingdom). The average ear swelling for each group of mice was calculated as:

$$AES = \Sigma \left[\frac{L_{post} + R_{post}}{2} - \frac{L_{pre} + R_{pre}}{2} \right] \div n$$

where L and R were the left and right ear thickness measurements from individual mice, and n was the number of mice per treatment group. The percent suppression was calculated as:

Percent Suppression

$$= \left[1 - \frac{Average\ Net\ Ear\ Swelling_{Irradiated}}{Average\ Net\ Ear\ Swelling_{Control}} \right] \times 100$$

The effects of the various diets on the solvent only responses (acetone vehicle replacing sensitizer solutions) and on the challenge only responses (mice challenged with 2,4-dinitrofluorobenzene without prior sensitization) were also examined.

Experimental design. All diets were fed for the duration of all experiments. To ensure that the various diets did not affect body weight or clinical health status, groups of 10 mice per diet were fed the various 5 and 20% fat diets for a period of 200 d. The mice were individually weighed periodically throughout this period.

The effect of the various dietary fats on the epidermal fatty acid composition was examined by feeding groups of three mice per diet the various 5 and 20% fat diets for 2 or 4 wk before killing. A group of three mice fed the nonpurified diet was also examined.

Initially, contact hypersensitivity was measured in mice fed the 20% fat diets with and without UVB irradiation. The various 20% fat diets were prefed to groups of 10 mice (5 irradiated, 5 controls) for either 2 or 4 wk before irradiation, and the contact hypersensitivity reaction was induced as described above. In all subsequent experiments, diets were prefed for 4 wk before irradiation. The effect of feeding the 20% fat diets on contact hypersensitivity was also measured with and without exposure to simulated solar ultraviolet radiation.

To establish whether a dose-response relationship existed between the proportions of sunflower oil and clarified butter fed and the degree of UVB suppression of the contact hypersensitivity reaction, 20% fat diets containing 0, 5, 10, 15, 17.5 or 20% sunflower oil (the balance of the 20% being clarified butter) were fed to groups of 10 mice (5 controls and 5 irradiated mice) per diet. Mice were exposed to UVB radiation and contact sensitized 7 and 8 d after the first UVB exposure and challenged 7 d after the first sensitization.

The various 5% fat diets were fed to groups of 10 mice (5 controls and 5 irradiated mice) per diet to examine the effect of these low fat diets on photoimmunosuppression due to UVB and solar simulated ultraviolet irradiation. Because the 20% fat experiments and the 5% fat experiments were performed independently from each other and considerable interexperimental variation was encountered with the contact hypersensitivity reaction, a valid comparison of the effects of high and low fat intake on the contact hypersensitivity reaction was not possible on the basis of the preceding experiments. To overcome this, groups of 10 mice (5 irradiated and 5 control mice) per diet were fed either the 5% sunflower oil diet or the 20% sunflower oil diet, irradiated with UVB and contact sensitized in the usual manner.

Statistical analyses. Data were analyzed by using one-way or two-way ANOVA. When appropriate, means were separated by using least significant difference test (SPIDA Version 6 software, Statistical Computing Lab., Macquarie University, Australia).

RESULTS

Effect of dietary fats on body weight and health status. The different dietary fats did not significantly affect body weight, weight gain or clinical health status of mice when fed for >200 d (**Fig. 1**). In addition, the variation in body weight within each dietary group over a 200-d feeding period was small. Because the mice were all housed and fed under identical conditions, this suggests that the average energy intake, and therefore the

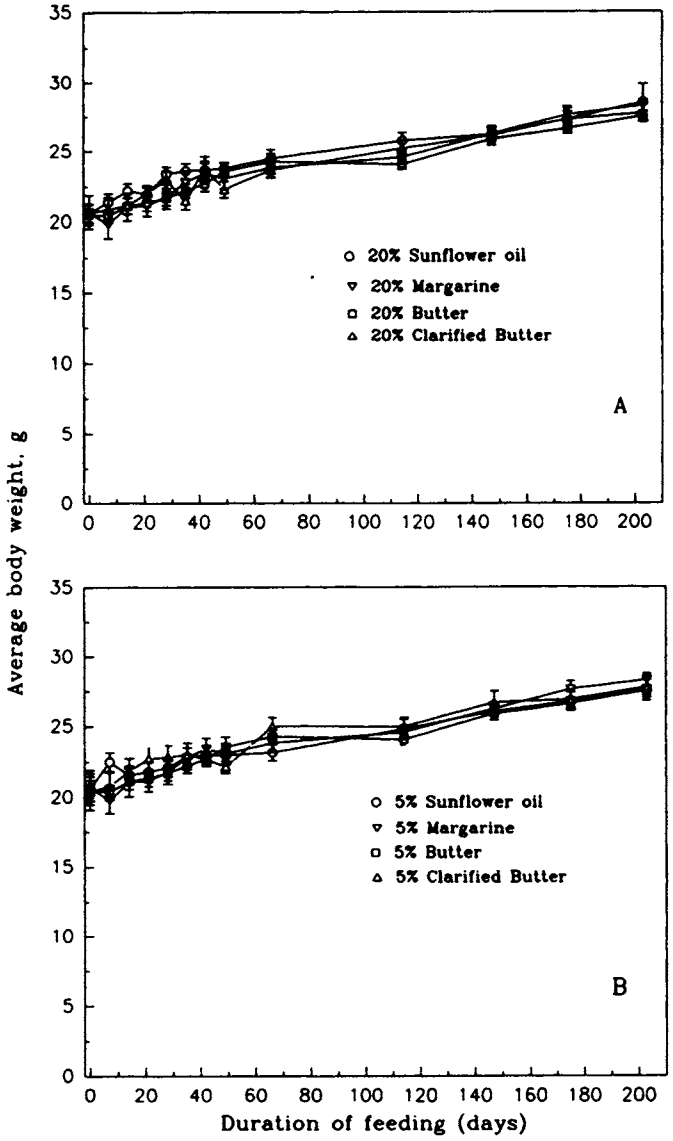

FIGURE 1 The effect of dietary fat on the mean (± SEM) body weight of groups of 10 mice. (*A*) 20% fat diets. (*B*) 5% fat diets.

intakes of the other components of the diets, did not differ significantly among the various dietary groups.

Effect of dietary fats on nonvolatile fatty acid composition of the epidermis. The epidermal nonvolatile fatty acid composition of nonpurified diet-fed mice is shown in **Table 4**. The composition was progressively altered by feeding the experimental 20% fat diets for 2 and 4 wk, and the major consistent changes were in the 18:2 fatty acid concentration. After 4 wk of feeding butter and clarified butter, the 18:2 levels of the epidermis were comparable and were significantly lower than in nonpurified diet-fed controls ($P < 0.05$.) In the mice fed sunflower oil or margarine, the 18:2 levels were significantly greater than in the nonpurified diet-fed mice ($P < 0.05$). The 18:2 concentrations in epidermis at 2 wk were intermediate for all diet groups except that fed the clarified butter, in which case the 18:2 level had already been reduced to a level similar to the concentration at 4 wk. The 18:2 concentration of the epidermis reflected the dietary intake of this fatty acid. Differences in other nonvolatile fatty acids present in the epidermis were relatively small. The 5% fat diets produced similar effects (data not shown).

Effect of dietary fats on the contact hypersensitivity reaction. The contact hypersensitivity reaction was characterised by an approximate doubling in average ear thickness in unirradiated control animals, a highly significant response ($P < 0.001$). The responses of unirradiated control animals fed the different diets containing either 5 or 20% fat were not significantly different (**Figs. 2–5**), and there were negligible effects of the diets on the solvent control or the challenge only control treatments (Figs. 2B and C).

Effect of duration of feeding and dietary fats on UVB-induced suppression of contact hypersensitivity. When the 20% fat diets were prefed for only 2 wk before UVB irradiation (Fig. 2A), there was no significant dietary fat-related difference in the contact hypersensitivity responses of the control mice or the irradiated mice, although the suppression of the reaction by UVB was significant ($P < 0.05$).

When the 5 and 20% fat diets were prefed for 4 wk before UVB irradiation (Figs. 2B and C), there was again no significant difference between the contact hypersensitivity responses of the control groups, irrespective of the dietary fat type. There was a significant ($P < 0.05$) reduction of the contact hypersensitivity responses of irradiated mice fed either sunflower oil or margarine compared with the nonirradiated controls. Irradiated mice fed butter or clarified butter also had significantly lower ($P < 0.05$) contact hypersensitivity reactions compared with the controls, but this reduction was significantly less ($P < 0.05$) than the UVB-suppressed responses in the mice fed sunflower oil or margarine. The contact hypersensitivity responses of mice fed sunflower oil or margarine were not statistically different from each other; the responses of mice fed butter and clarified butter were also not significantly different from each other. Thus 4 wk of feeding the various dietary fats before irradiation was necessary to modify the UVB-induced suppression of contact hypersensitivity, whereas 2 wk was insufficient.

Effect of dietary fats on simulated solar ultraviolet radiation-induced suppression of the contact hypersensitivity reaction. When the 20 and 5% fat diets were fed for 4 wks before simulated solar ultraviolet irradiation (**Fig. 3**), the pattern of effects of dietary fat on the contact hypersensitivity response was similar to that induced by UVB irradiation. In both experiments, the diets did not affect the contact hypersensitivity reactions of the unirradiated groups, and irradiated mice fed butter or clarified butter were not significantly immunosuppressed compared with the unirradiated control animals. The contact hypersensi-

TABLE 4

Composition of nonvolatile fatty acids in the epidermis of mice fed 20% fat diets for 2 or 4 weeks compared with nonpurified diet-fed mice[1]

Fatty acid	Nonpurified diet	Butter		CB[2]		SO[2]		Margarine	
		2 wk	4 wk	2 wk	4 wk	2 wk	4 wk	2 wk	4 wk
	mol/100 mol								
10:0	ND	1.02 ± 0.51	ND	0.7 ± 0.06	1.00 ± 0.16	ND	ND	1.09 ± 0.25	ND
12:0	0.46 ± 0.15	0.46 ± 0.01	1.89 ± 0.05	1.31 ± 0.04	1.54 ± 0.06	3.50 ± 0.8	0.27 ± 0.04	1.11 ± 0.19	0.80 ± 0.19
14:0	12.75 ± 0.98	3.26 ± 0.64	7.86 ± 0.11	6.53 ± 0.15	6.86 ± 0.02	1.00 ± 0.32	1.23 ± 0.23	4.56 ± 0.74	3.27 ± 0.77
16:0	17.65 ± 1.21	20.62 ± 1.65	29.25 ± 0.43	28.00 ± 0.91	28.55 ± 0.28	13.71 ± 0.05	14.97 ± 0.26	20.33 ± 1.61	19.83 ± 1.39
16:1	6.59 ± 0.10	5.98 ± 1.42	5.72 ± 0.25	7.76 ± 0.55	7.29 ± 1.29	4.93 ± 0.19	4.06 ± 0.26	4.88 ± 0.86	2.77 ± 0.26
18:0	5.76 ± 0.29	7.63 ± 0.07	9.22 ± 0.31	7.54 ± 0.50	6.86 ± 0.75	5.61 ± 0.17	4.90 ± 0.40	8.61 ± 0.26	6.75 ± 0.40
18:1	23.97 ± 0.87	27.91 ± 1.16	31.74 ± 0.34	29.36 ± 0.60	30.72 ± 0.55	23.42 ± 0.39	25.36 ± 0.27	28.92 ± 0.27	30.80 ± 0.22
18:2	21.12 ± 0.35	19.14 ± 4.26	8.96 ± 0.20[b]	9.51 ± 0.78	10.12 ± 2.03[b]	35.13 ± 0.54	36.88 ± 0.63[a]	19.73 ± 2.21	28.22 ± 1.80[a]
20:1	9.59 ± 0.74	7.71 ± 0.68	3.47 ± 0.09	5.32 ± 0.47	3.83 ± 0.03	5.68 ± 0.05	3.74 ± 0.49	5.21 ± 0.60	3.74 ± 0.29
20:2	4.74 ± 0.62	1.50 ± 0.01	3.46 ± 1.16	1.11 ± 0.05	0.75 ± 0.04	3.76 ± 0.16	3.62 ± 0.38	1.77 ± 0.25	1.40 ± 0.28
20:3	10.68 ± 0.59	4.80 ± 0.65	0.95 ± 0.08	2.87 ± 0.35	2.5 ± 0.06	4.56 ± 0.86	5.06 ± 0.14	4.93 ± 0.70	2.4 ± 0.45

[1] Results are means ± SEM of three separate analyses and are expressed as mol of fatty acid/100 mol of total fatty acids.
[2] CB = clarified butter; SO = sunflower oil; ND = not detected.
[a] Significantly greater ($P < 0.05$ than the nonpurified diet-fed group and the butter- and clarified butter-fed groups at 4 wk.
[b] Significantly less ($P < 0.05$) than the nonpurified diet-fed group but not different than one another.

tivity responses of irradiated mice fed the sunflower oil and margarine diets were not significantly different; however, these groups had significantly lower ($P < 0.05$) contact hypersensitivity reactions compared with both the unirradiated control groups and the irradiated mice fed the saturated fats.

Effect of mixed fat diets on UVB suppression of the contact hypersensitivity reaction. In irradiated animals, there was a strong linear relationship between the average contact hypersensitivity response and the proportion of sunflower oil or clarified butter fed ($r = 0.98$, $r^2 = 0.95$; **Fig. 5**). The larger the proportion of sunflower oil in the diet, the greater the UVB suppression of the average ear swelling response. Photoimmunosuppression was significant in all irradiated groups ($P < 0.05$). The mixed fats did not significantly affect the contact hypersensitivity response of unirradiated animals.

Effect of high and low sunflower oil diets on UVB suppression of the contact hypersensitivity reaction. There was no significant difference in the average ear swelling responses of the unirradiated mice fed the 20% or the 5% sunflower oil diets ($P > 0.05$; Fig. 5). All irradiated mice had significant suppression of the average ear swelling response compared with the unirradiated controls ($P < 0.05$). The level of polyunsaturated fat in the diet did not significantly affect the ear swelling responses of irradiated mice ($P < 0.05$).

DISCUSSION

The above results demonstrate that butter and clarified butter, compared with margarine and sunflower oil, protected against the systemic suppression of contact hypersensitivity induced by moderate exposure to UVB radiation or simulated solar ultraviolet radiation. The effects were not due to modulation of the contact hypersensitivity reaction per se because the different fat sources, whether fed as the sole fat or as part of a mixed fat diet, had no effect on the reaction in unirradiated control mice.

Because it is uncertain how the immunomodifying effects of experimental UVB irradiation in mice might relate to possible effects of the full solar ultraviolet spectrum in humans, it is relevant that we have observed the same dietary fat dependence of the contact hypersensitivity reaction in mice with both ultraviolet light sources.

The immunoprotective effect of butter and clarified butter was apparent when the fats were fed not only as the sole dietary fat source but also when mixed fat diets were fed, combining sunflower oil with clarified butter. It appears, therefore, that alteration of the balance of fats in the human diet might provide the opportunity to similarly modify immune responsiveness after solar ultraviolet exposure.

The modulation of this response was apparent after 4 wk of feeding the altered dietary fats but not after only 2 wk of feeding. When fed as the sole dietary fat, butter and clarified butter progressively reduced the 18:2 concentration of the epidermis, in agreement with the observation of a linear association between linoleic acid levels in the epidermal phospholipids of SENCAR mice and dietary linoleic acid intake (Leyton et al. 1991). It is relevant that after 2 wk of feeding these fats, there was no reduction in epidermal 18:2 by butter, but

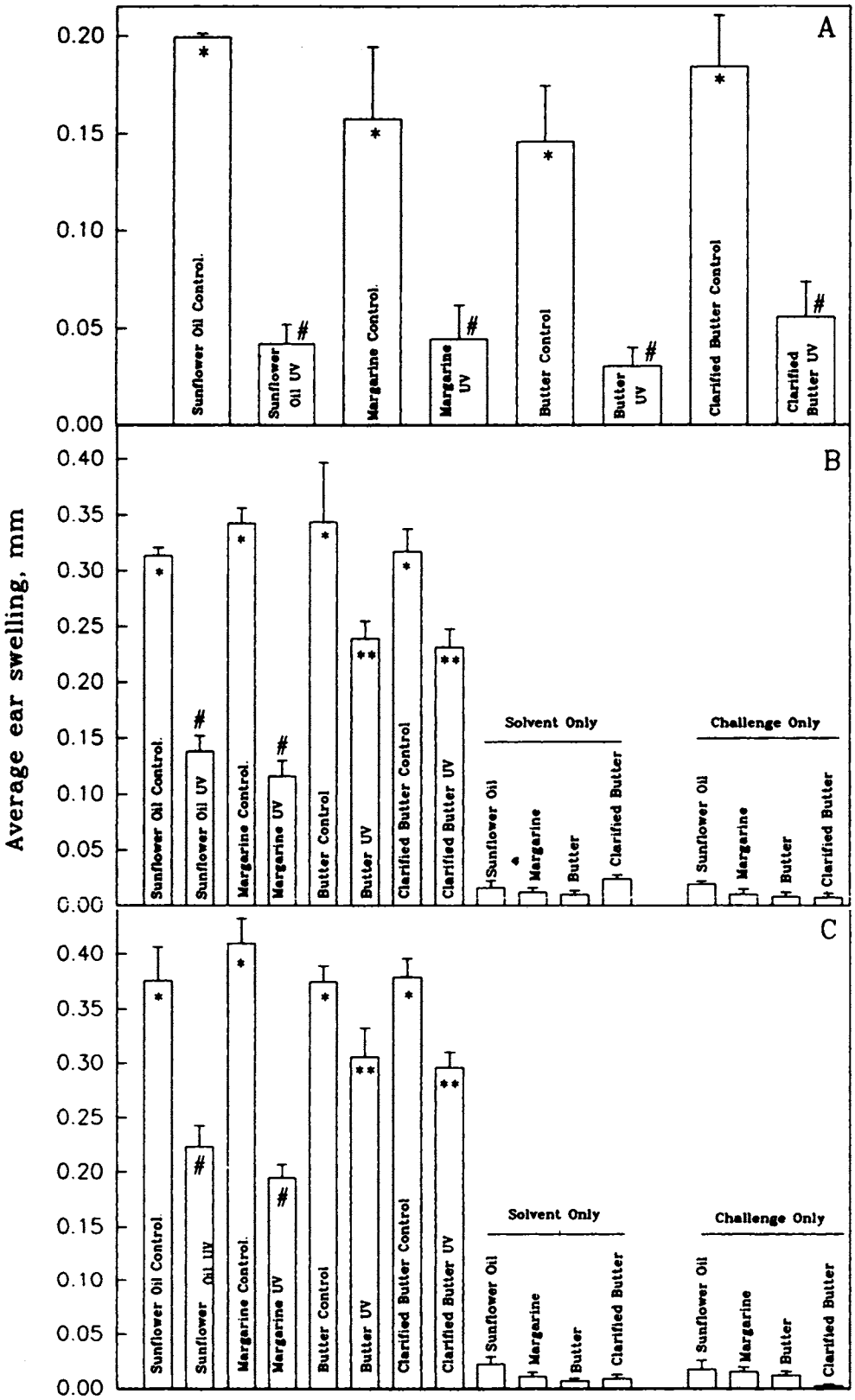

FIGURE 2 The effect of dietary fat on the contact hypersensitivity response of groups of five mice, with and without exposure to UVB radiation, expressed as the mean (± SEM) ear swelling response to hapten challenge. (*A*) 20% fat diets were prefed for 2 wk before UVB irradiation; (*B*) 20% fat diets were prefed for 4 wk before UVB irradiation; (*C*) 5% fat diets were prefed for 4 wk before irradiation. Solvent only and challenge only controls indicate negligible ear swelling. Within each panel, matching superscripts indicate values not differing significantly; differing superscripts indicate significantly different values ($P < 0.05$).

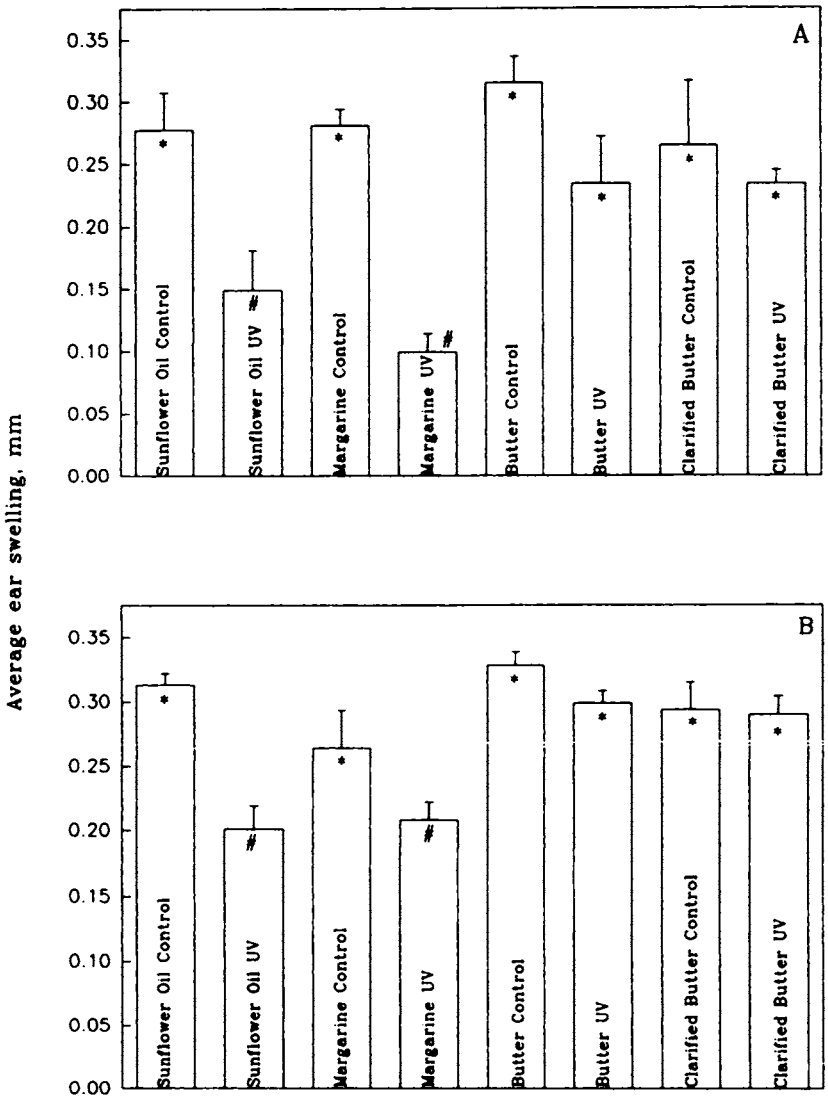

FIGURE 3 The effect of dietary fat on the contact hypersensitivity response in groups of five mice, with and without exposure to solar simulated ultraviolet radiation, expressed as the mean (± SEM) ear swelling response to hapten challenge. (*A*) 20% fat diets; (*B*) 5% fat diets. Diets were prefed for 4 wk. Within each panel, matching superscripts indicate values not differing significantly; differing superscripts indicate significantly different values ($P < 0.05$). The values are not comparable between panels, because the experiments were performed at different times.

by clarified butter the reduction in 18:2 was already marked. The contact hypersensitivity responses in UVB-irradiated mice at this time were similarly suppressed in butter-fed mice as in mice fed sunflower oil and margarine, but a slightly reduced suppression was evident in clarified butter-fed mice. Four weeks of feeding resulted in marked alteration in the epidermal fatty acid composition, particularly a reduction of 18:2 by butter and clarified butter, and an increase by sunflower oil and margarine, which was reflected by the degree of photoimmunosuppression observed. Thus the major alteration in epidermal fatty acid composition achieved by the different dietary fats, that of 18:2 content, seemed to relate to the susceptibility to photoimmunosuppression. Notably, there was a linear relationship between the proportion of sunflower oil in the diet (rich in linoleic acid) and the degree of photoimmunosuppression when mixed sunflower oil/clarified butter diets were fed.

Linoleic acid forms a significant component of the lipids making up the lamellar bodies of the stratum corneum (Elias et al. 1980). Because the composition of the lamellar bodies of the skin affects the pharmacokinetics of compounds in the stratum corneum and because a key mediator of photoimmunosuppression, *cis*-urocanic acid, is located predominantly in the stratum corneum (DeFabo and Noonan 1983), it is conceivable that alteration of the lipid composition may have altered the pharmacokinetics of this mediator with resultant effects on photoimmunosuppression.

No difference was evident between the effects of

butter and clarified butter, nor between margarine and sunflower oil, supporting the suggestion that the essential regulating factor in the butter/clarified butter or margarine/sunflower oil was the composition of the predominant lipids, or other minor fat-soluble components that will be considered here.

The butter clarification process reduced the content of nonfat solids in butter by a factor of approximately 30. Because there was no difference between the effects produced by feeding butter or clarified butter, the observed protective effects were not associated with the nonfat solids present in the butter. Similarly, because there was no difference in the effects yielded by feeding sunflower oil or margarine, the presence of partially hydrogenated cottonseed oil, *trans*-fatty acids (approximately 9.5 mol/100 mol *trans*-18:1), lecithin, β-carotene, vitamins A and D, milk-derived nonfat solids and salt in the margarine had no effect on photoimmunosuppression.

The β-carotene concentration of the fats differed. β-carotene intake and/or plasma concentration have been linked to the modulation of a variety of T cell and T cell-associated functions in rats, mice and humans, and β-carotene has also been demonstrated to protect from ultraviolet radiation-induced suppression of delayed type hypersensitivity in humans (Fuller et al. 1992, Jyonouchi et al. 1993). Other studies demonstrated that dietary β-carotene supplementation of mice receiving adequate dietary vitamin A did not affect photoimmunosuppression and did not significantly alter serum retinol levels, so the potential photoimmunoprotective role of β-carotene is unclear

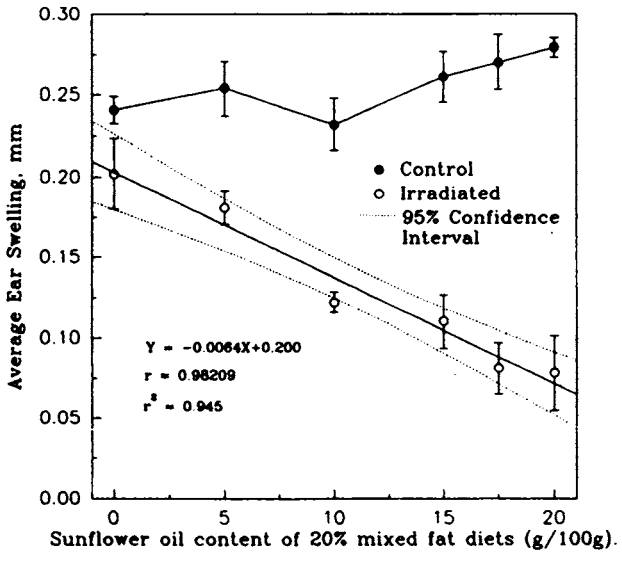

FIGURE 4 The effect of 20% mixed fat diets (prefed for 4 wk) on the contact hypersensitivity response in groups of five mice, with and without exposure to UVB radiation, expressed as the average (± SEM) ear swelling response to hapten challenge. The diets contained increasing proportions of sunflower oil, the balance of the 20% fat content being clarified butter.

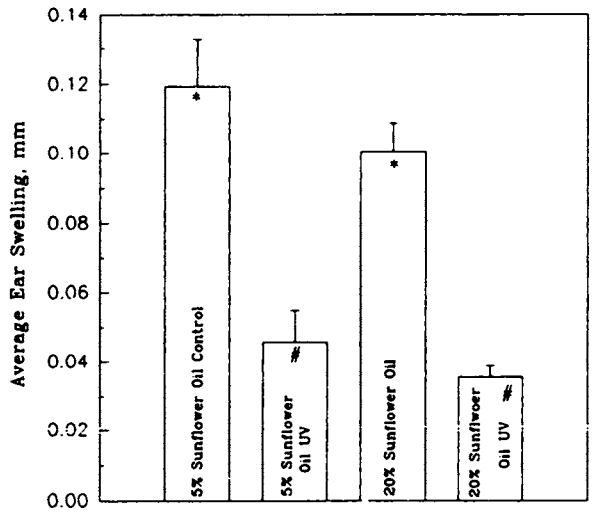

FIGURE 5 The effect of high (20%) and low (5%) sunflower oil diets (pre-fed for 4 wk) on the contact hypersensitivity response in groups of 5 mice, with and without exposure to UVB radiation, expressed as the mean (± SEM) ear swelling response to hapten challenge. Matching superscripts indicate values not differing significantly; differing superscripts indicate significantly different values ($P < 0.05$).

(Noonan et al. 1994). The sunflower oil contained no β-carotene and the margarine contained 6.6 mg/100 g, yet the effects on photoimmunosuppression were comparable. The margarine contained a level of β-carotene similar to that of the butter, yet the effects of these different fats on photoimmunosuppression were disparate. It is therefore concluded that the β-carotene concentration of the fats was not the primary immunomodulating factor in this study. This is consistent with a report that oral or topical retinoids did not affect photoimmunosuppression or the contact hypersensitivity reaction in animals fed vitamin A-deficient or normal diets (Ho et al. 1991).

The tocopherol concentration of the different fats varied. Tocopherols also absorb strongly in the UVB range and may produce an ultraviolet radiation screening effect in the epidermis (Fryer 1993). The total tocopherol concentration of the butter and clarified butter was ~6–8% that of the margarine and sunflower oil, yet protection from photoimmunosuppression was seen in mice fed butter or clarified butter. Photoimmunosuppression was significantly greater in the animals fed margarine and sunflower oil, suggesting the possibility that the increased tocopherol intake may be detrimental in terms of photoimmunosuppression. However, the same pattern of immunosuppression was seen in mice fed the 5 and 20% fat diets, suggesting that a 75% lower intake of tocopherols derived from the different fats in this study did not affect the observed pattern of photoimmunosuppression. The dietary intakes of tocopherols in these experiments do not logically explain the observed effects.

Lipid hydroperoxides were not observed in the differ-

ent fats and therefore did not contribute to the phenomena observed in this study. Vitamin D was present at similar low levels in all of the dietary fats used in this study. Vitamin D is unlikely to be relevant in photoimmunological reactions because the action spectrum of its photochemical reaction in skin differs substantially from the known action spectrum of photoimmunosuppression (De Fabo and Noonan 1983).

The use of butter or clarified butter as the sole dietary fat was associated with an approximate 64 to 75% reduction in the 18:2 level of the epidermis compared with mice fed the polyunsaturated fats. Such an alteration in the availability of this eicosanoid precursor in the epidermis could be postulated to have affected the eicosanoid requirement for both the contact hypersensitivity response and the response to UV irradiation. If the eicosanoid system was affected in this study, it was affected in a manner that did not alter the edema response to hapten re-exposure, because the different dietary fats and the change in skin 18:2 levels did not affect the contact hypersensitivity reaction per se (as measured by the ear thickening response which is predominantly, but not totally, due to edema). The inhibition of prostaglandin synthesis by indomethacin was demonstrated not to affect the late contact hypersensitivity reaction to picryl chloride, which would be consistent with our findings if our diets altered eicosanoid synthesis, whereas platelet activating factor antagonists have been demonstrated to inhibit this reaction, implying that noncyclooxygenase dependent mediators are more important in the production of skin edema after reexposure to hapten (Lavaud et al. 1991). However, other studies demonstrated the reverse: indomethacin and other cyclooxygenase inhibitors interfere with the contact hypersensitivity reaction (Nakamura et al. 1988).

Prostaglandin E_2 is produced in the epidermis after UVB irradiation and has been proposed as one of the effectors responsible for the immunological effects associated with UVB exposure (Chung et al. 1986, Jun et al. 1988). Alteration of the availability of arachidonic acid precursors could therefore be postulated to affect the immunological consequences of ultraviolet radiation exposure. Alteration of fatty acid eicosanoid precursors in the epidermis and modulation of the eicosanoid and immune responses to ultraviolet radiation by consumption of diets rich in (n-3) polyunsaturated fatty acids was previously reported (Fischer and Black 1991). The dietary fats in our study contained predominantly (n-6) unsaturated fatty acids, but the reduction in arachidonic acid precursors was similar.

On the other hand, the wavelengths of light, particularly in the UVA portion of the spectrum, that are known to induce skin prostaglandin production differ significantly from the currently accepted action spectrum for photoimmunosuppression (De Fabo and Noonan 1983). This could be explained by the postulate that prostaglandins are second-order mediators of photoimmunosuppression (De Fabo and Noonan 1983). Recently, a strong case for the role of DNA damage in the production of photoimmunosuppression has been made (Kripke et al. 1994, Wolf et al. 1994). The action spectrum for DNA damage in skin differs substantially from the currently accepted action spectrum for photoimmunosuppression (De Fabo and Noonan 1983). This paradox is yet to be clarified; however, it implies that the currently accepted action spectrum for photoimmunosuppression may not take into account all the potential mechanisms of its induction.

The role of the lipoxygenase system in photoimmunosuppression is less well established. Because 18:2 fatty acids are also precursors for this cascade, modulation of lipoxygenase products may also have occurred in this study.

Both β-carotene and the tocopherols are efficient quenchers of lipid peroxide-driven reactions in the mouse epidermis (Mathews-Roth et al. 1986, Shindo et al. 1993). The photochemical generation of lipid peroxides from unsaturated fatty acids in the epidermis is one potential mechanism of multiple organelle and DNA damage within cells and is a proposed mechanism of photoimmunosuppression (Mathews-Roth 1986, Picardo et al 1991), which would be consistent with our results in which an increased polyunsaturated fatty acid concentration of the diet has enhanced photoimmunosuppression. However, because our results are not consistent with a role for β-carotene or tocopherols in protecting against photoimmunosuppression, lipid peroxides do not appear to be the primary mediators, a conclusion also reached by De Fabo and Noonan (1983) based on action spectrum data.

Dietary lipids have been demonstrated to modify the lipid composition of lymphocyte cell membranes, and these alterations have been claimed to be immunomodulatory (Erickson 1986, Mertin and Mertin 1988). If the dietary fats in our study altered lymphocyte lipid composition in a functionally important manner, modulation of the contact hypersensitivity reaction in unirradiated animals would be expected. This was not observed, suggesting that if alteration of lymphocyte lipid composition occurred, it was only of any functional consequence after exposure to ultraviolet radiation. Membrane lipid alteration may indirectly alter lymphocyte function via altered membrane receptor function (Erickson 1986, Mertin and Mertin 1988). Splenic receptors modified by an epidermally originating ultraviolet radiation photoproduct or messenger may be involved in systemic photoimmunosuppression (Noonan and De Fabo 1993).

Oxidized cholesterol derivatives are potent immunomodulatory agents in vitro (Humphries and McConnell 1979). Cholesterol is susceptible to atmospheric oxidation, even in the dark and at low temperatures (Humphries and McConnell 1979). Because the pattern

of dietary fat and photoimmunosuppression was comparable in mice fed both the 20 and 5% fat diets, a role for these compounds in the effects observed in this study is unlikely.

In summary, this study demonstrates the phenomenon of dietary fat alteration of the immunological consequences of ultraviolet radiation exposure. Feeding mice butter or clarified butter provided significant protection against photoimmunosuppression compared with sunflower oil or margarine. The results of this study strongly implicate the fatty acid composition of the dietary fats as the photoimmunologically modulating factor(s). The associated modification of the epidermal fatty acid composition may be a major factor; however, the precise mechanism of how this immunomodulation is achieved remains an enigma.

ACKNOWLEDGMENTS

We thank Lyn Blyth, Leslie Gabor, Rosalind Manny and Georgina Philips for excellent mouse husbandry, Dallas McMillan for careful laboratory assistance and Graham Bailey for assistance with the gas-liquid chromatography analyses. We are grateful to Caroline Kerr and John Mercer, Department of Animal Science, University of Sydney, University of Sydney, for helpful discussions.

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