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Research Article

Female B/W mice spontaneously develop an autoimmune disease that is similar to systemic lupus erythematosus. Antibodies to doublestranded DNA (dsDNA) and antinuclear antibodies develop in aging animals; death from immune complex-mediated glomerulonephritis occurs from 8 to 12 mo of age. It has been reported that prostaglandin (PG)E_1 treatment of such mice prolongs survival. In the present study, four groups of female B/W mice were studied beginning at 6-11 wk of age on the following regimens: (*a*) a synthetic diet that contained 20% safflower oil, (*b*) a standard laboratory chow diet, (*c*) a standard diet together with injections of PGE_1, and (*d*) an essential fatty acid-deficient synthetic diet that contained 20% coconut oil. All animals were tested monthly for antinuclear antibodies and anti-dsDNA. Kidney tissue was obtained for light and immunofluorescence microscopy when animals were dying. All disease manifestations were altered strikingly in the essential fatty acid (EFA)-deficient animals. Intermediate benefit was seen in PGE_1-treated animals. 7% of the control animals and 18% of safflower oil-fed animals survived to 10 mo. In contrast, the PGE_1-treated and EFA-deficient mice had a similar survival rate (78-88%). At age 16 mo, 78% of EFA-deficient mice and 45% of PGE_1-treated mice were alive. 25% of the PGE_1-treated and 55% of the EFA-deficient animals survived to 20 mo. Serum anti-dsDNA appeared at age 5 mo in […]

Prevention of Glomerulonephritis and Prolonged Survival in New Zealand Black/New Zealand White F_1 Hybrid Mice Fed an Essential Fatty Acid-deficient Diet

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ABSTRACT Female B/W mice spontaneously develop an autoimmune disease that is similar to systemic lupus erythematosus. Antibodies to double-stranded DNA (dsDNA) and antinuclear antibodies develop in aging animals; death from immune complex-mediated glomerulonephritis occurs from 8 to 12 mo of age. It has been reported that prostaglandin (PG)E_1 treatment of such mice prolongs survival. In the present study, four groups of female B/W mice were studied beginning at 6–11 wk of age on the following regimens: (*a*) a synthetic diet that contained 20% safflower oil, (*b*) a standard laboratory chow diet, (*c*) a standard diet together with injections of PGE_1, and (*d*) an essential fatty acid-deficient synthetic diet that contained 20% coconut oil. All animals were tested monthly for antinuclear antibodies and anti-dsDNA. Kidney tissue was obtained for light and immunofluorescence microscopy when animals were dying. All disease manifestations were altered strikingly in the essential fatty acid (EFA)-deficient animals. Intermediate benefit was seen in PGE_1-treated animals. 7% of the control animals and 18% of safflower oil-fed animals survived to 10 mo. In contrast, the PGE_1-treated and EFA-deficient mice had a similar survival rate (78–88%). At age 16 mo, 78% of EFA-deficient mice and 45% of PGE_1-treated mice were alive. 25% of the PGE_1-treated and 55% of the EFA-deficient animals survived to 20 mo. Serum anti-dsDNA appeared at age 5 mo in safflower oil-fed and control animals, but not until 9 and 12 mo for PGE_1-treated and EFA-deficient animals, respectively. All kidneys from 7- to 9-mo-old safflower oil-fed and control animals and the majority of kidneys from PGE_1-treated animals were abnormal by light and immunofluorescence microscopy. Kidneys from EFA-deficient animals were essentially normal at 10 mo. At 13 mo, all PGE_1-treated animals examined had significant kidney involvement, whereas none of the EFA-deficient animals had glomerulonephritis. These findings demonstrate that an EFA-deficient diet has a beneficial effect on murine lupus erythematosus.

INTRODUCTION

The New Zealand Black/New Zealand White F_1 (B/W)[1] hybrid mouse is an excellent animal model for the study of human systemic lupus erythematosus (1, 2). These mice develop a number of immunologic abnormalities, e.g., anti-DNA and antinuclear antibodies (ANA) and hypergammaglobulinemia. They also have circulating immune complexes and immunoglobulin deposition in the renal glomerulus and along the dermal-epidermal junction (3). The progression of the skin and kidney immune deposits have been correlated (4). The maximal degree of severity of glomerulonephritis (GN) is attained at 9 mo of age and causes death of most animals by 12 mo of age (1, 2, 5).

In a recent series of experiments, Zurier and coworkers (6, 7) demonstrated a beneficial effect of prostaglandin (PG)E_1 treatment of these animals on survival (6) and renal disease (7). Using large doses of

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[1] *Abbreviations used in this paper*: ANA, antinuclear antibody; B/W, F_1 hybrid of New Zealand Black × New Zealand White; dsDNA, double-stranded DNA; EFA, essential fatty acid; GN, glomerulonephritis.

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PGE_1, these workers found a significant reduction in immunoglobulin deposition in the kidneys and in proliferative GN. PGE_1 treatment did not, however, prevent the development of antibodies to nuclear material.

In the present investigation using B/W mice, we examined the effects of diets containing a high concentration of linoleic acid, thus rich in essential fatty acid, and diets deficient in essential fatty acid, which are, therefore deficient in potential PG precursor. The first group was fed safflower oil that contained 78% linoleic acid. Linoleic acid is a precursor of both eicosa-8,11,14-trienoic (dihomo-γ-linolenic) and eicosa-5,8,11,14-tetraenoic (arachidonic) acids which can be converted to prostaglandins or prostaglandin-related compounds such as thromboxanes, prostacyclins, or leukotrienes. A second group of animals was fed a normal chow diet. The third group of animals was fed the chow diet and was injected with PGE_1 in doses identical to those used by Zurier and co-workers (6, 7). The fourth group was fed a diet deficient in essential fatty acids that contained coconut oil (<1% linoleic acid).

In these studies we found markedly prolonged life, reduced severity of GN and very much lower levels of ANA and anti-DNA antibodies in the animals fed an essential fatty acid (EFA)-deficient diet. Safflower oil feeding had no apparent beneficial effect on the severity of GN or survival. PGE_1 treatment caused prolonged survival but was less effective than coconut oil feeding in reducing or preventing autoantibody production or the development of GN.

METHODS

Animals. The female B/W animals used were from our own colony that was established with mice obtained from Dunedin, New Zealand. The animals were entered into the study when the mice were 6–7 wk of age with the exception of the untreated control group which was entered into the study at 10–11 wk of age. The groups consisted of 11 mice fed a synthetic diet containing 20% safflower oil, 14 untreated control mice fed a chow diet, 11 mice fed a chow diet and injected with PGE_1 intraperitoneally, and 12 mice fed a synthetic diet containing 20% coconut oil. The mice were killed when obviously dying, but tissues were not available for study in a number of animals that died. Three animals each in the PGE_1-treated and EFA-deficient groups were killed at age 13 mo. Each animal was weighed five times a week for the first 2 mo, and weekly thereafter.

Diets. Colony control animals and those injected with PGE_1 were fed standard laboratory chow ad lib. The diets for the groups fed safflower or coconut oil were identical except for the individual oil added. Diets for the latter two groups consisted of casein (25%), dextrins (35%), sucrose (15%), a complete vitamin mixture (2%), a complete mineral mixture (3%) (8), and the oil (20% [either safflower or coconut]). Diets were given ad lib. The analyses of the fatty-acid composition of the safflower and coconut oils used in the present study are given in Table 1. The methyl esters of the fatty acids were prepared according to the procedure of Morrison and Smith (9). The fatty-acid methyl esters were dissolved in carbon disulfide and were separated by gas-liquid chromatography using a Hewlett-Packard model 5830A gas chromatograph with an integrator (utilizing columns of 10% SP-2330 on 100/120 chromosorb W at 200°C, Hewlett-Packard Co., Palo Alto, Calif.). The amounts of eluted fatty-acid methyl esters were computed using hepta-decanoic acid as an internal standard.

TABLE I
Fatty Acid Composition of Safflower and Coconut Oil (EFA-deficient) Diets

No. C: no double bonds	Safflower oil	Coconut oil
	mole %	*mole %*
8:0	-0-	9.9
10:0	-0-	7.1
12:0 (Lauric)	-0-	53.0
14:0 (Myristic)	-0-	15.7
16:0 (Palmitic)	7.3	6.5
18:0 (Stearic)	2.3	1.8
18:1 (Oleic)	12.5	4.2
18:2 (Linoleic)	77.9	<1.0

PGE_1 treatment. PGE_1 was provided by Dr. John E. Pike of the Upjohn Co., Kalamazoo, Mich. The PGE_1 was dissolved in a phosphate-buffered saline:ethanol (90:10) solution and adjusted to pH 7.2 before injection. Animals were injected with 200 μg PGE_1 intraperitoneally twice daily, 5 d each week.

Plasma. Blood was obtained from the retrobulbar venous plexus (~200 μl) of the mice each month and the harvested plasma was stored at −70°C until assayed.

Preparation of tissues. Animals were examined frequently. When the mice become moribund, they lose weight, develop pitting edema, hunched posture, decreased activity, and rapid, shallow breathing. At this time, the animals were killed; their kidneys were removed and prepared for study. Portions of kidney tissue were fixed in formalin for light microscopy and other portions were immersed in Tissue-Tek II embedding medium (Lab-Tek Div., Miles Laboratories Inc., Naperville, Ill.), frozen, and stored at −70°C.

Measurement of fatty acids in kidney and liver tissue. The lipids were extracted from the tissues according to the procedure of Folch et al. (10). The fatty acids were assayed by the procedure described above.

Immunofluorescence. Kidney tissues obtained when the mice were killed were examined for the presence of immunoglobulin deposits by the direct immunofluorescence technique. Fresh tissue was frozen immediately and embedded in Tissue-Tek II (Lab-Tek Products). Cryostat sections (4 μm) were stained with fluorescein isothiocyanate-conjugated anti-mouse IgG and IgM (Meloy Laboratories Inc., Springfield, Va.), washed three times, and mounted in buffered glycerol. Tissue sections were examined with Leitz Orthoplan fluorescence microscope equipped with epi-illumination (E. Leitz, Inc., Rockleigh, N. J.) An HBO 100 ultra-high-pressure mercury lamp and K490 and K510 filters (Osram, Munich, Germany) were used. Photomicrographs were taken with a Leitz Orthomat camera on Tri-X Pan black and white film, ASA 400 (Eastman Kodak Co., Rochester, N. Y.). Dr. Hurd examined all tissue sections without knowledge of the treatment received by the animal. They were graded 0 through 4+ according to distribution and intensity of fluorescence. Separate

scores were given for capillary loops, mesangium, and the overall appearance of the glomerulus.

Light microscopy. Formalin-fixed tissue was embedded in paraffin. Hematoxylin, eosin, and periodic acid Schiff-stained tissue sections were prepared from paraffin blocks for routine light-microscopic examination. All tissue sections were coded and examined without knowledge of treatment category. Histologic abnormalities of the kidney were scored on the basis of six separate categories: (*a*) glomerular cellularity, (*b*) alterations in mesangial matrix, (*c*) presence of epithelial crescents, (*d*) fibrinoid changes and presence of wire looplike alterations, (*e*) amount of perivascular and interstitial mononuclear cell infiltration, and (*f*) percentage of glomeruli that were hyalinized. An overall score was derived from an average of the first five of the above categories (percent of glomeruli that were hyalinized was expressed separately).

Measurement of antibodies. Antinuclear antibody was measured using K-B carcinoma cells on slides as substrate (Electro-Nucleonics, Inc., Fairfield, N. J.). Antibodies to native DNA were assessed by using the *Crithidia luciliae* (American Type Culture Collection 14765, Rockville, Md.) indirect immunofluorescence technique as described (11). In this assay, the kinetoplast (a giant mitochondrion containing histone-free dsDNA) of *C. luciliae* is used as the dsDNA substrate. Kinetoplast fluorescence after exposure to test sera followed by fluorescein isothiocyanate-conjugated goat antisera to mouse immunoglobin indicates the presence of anti-dsDNA. Serum antibody concentration was determined by serial dilutions to the point of disappearance of kinetoplast fluorescence. Kinetoplast fluorescence following the application of undiluted test serum was considered positive, because undiluted mouse serum from a large number of nonautoimmune strains have been consistently negative. The class composition of anti-dsDNA antibodies in age-matched pooled sera from each study group was evaluated by using monospecific anti-mouse IgG or IgM conjugates in the final step of the procedure.

Vaginal smears. To ascertain whether the estrus cycles of the mice were normal, smears of vaginal cells were prepared. The tip of a small medicine dropper was filled with saline and inserted into the vaginal opening. One drop of the resulting cell suspension was placed on a slide with a drop of methylene blue and a cover slip. Cells were observed at both low and high magnification to ascertain the stages of maturation and estrus (12).

RESULTS

Weights. The average weight gain for each of the four groups of animals (Fig. 1) was similar. The greatest weight gain was found in animals fed safflower oil. However, initial weights were slightly higher in this group. The weights of the untreated animals, the PGE_1-treated, and the coconut oil-fed animals were similar. The percent weight gain for each group, as compared with the initial weight, was greatest for the safflower oil-fed group and least for the untreated control group (data not shown). It should be noted, however, that the untreated group was not entered into the study until the mice were 10–11 wk old.

Measurement of fatty acids in kidney and liver tissue. Samples of kidney and liver tissue were obtained from the animals on the coconut oil diet and

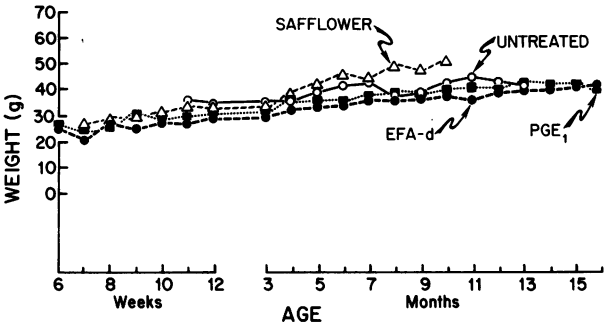


FIGURE 1 Weights (in grams) of B/W mice.

from the PGE_1-injected animals that were killed at age 13 mo. The lauric acid content of kidney and liver tissue of the PGE_1-treated animals was <0.2% of the total fatty-acid content of these tissues. The lauric acid contents of the kidney and liver tissue of the coconut oil-fed animals were 8.2 and 1.6%, respectively. The linoleic and arachidonic acid compositions (expressed as mole percent of the total fatty acid fraction) of kidney tissues of the PGE_1-injected mice were 24.0 and 4.7, respectively. Similar values for arachidonic acid content of kidney tissue from an 11-mo-old NZB, a 13 mo-old-NZB, and a 13-mo-old CBA/J mouse were found. For the coconut oil-fed mice the values were 3.6 and 2.5. A similar decrease in the linoleic and arachidonic acid composition was also found in lipid extracts of liver tissue obtained from the coconut oil-fed mice compared to that of the PGE_1-injected animals.

Survival. The percent survival of the four groups of animals as a function of age is shown in Fig. 2. The lifespans of the untreated control group and the safflower oil-fed animals were similar. Safflower oil feeding afforded no protection to the animals; all of the safflower oil-fed animals were dead by 10.5 mo of

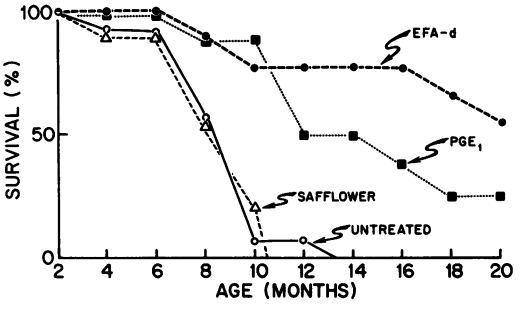


FIGURE 2 Percent survival of all four groups of animals. PGE_1-treated vs. safflower oil-fed or untreated groups ($P < 0.02$). EFA-d vs. safflower oil-fed or untreated groups ($P < 0.02$).

age. All of the control animals were dead by 10 mo of age with the exception of one animal that survived until age 13 mo.

Both the PGE_1-treated and coconut oil-fed animals had a markedly prolonged survival ($P < 0.02$). The length of survival of the coconut oil-fed animals was, however, longer than that of the PGE_1-treated animals. 50% of the PGE_1-treated animals were alive at age 14 mo and 38% were alive at age 16 mo. In comparison, 78% of the coconut oil-fed mice were alive at 16 mo of age. 25% of the PGE_1-treated and 55% of the coconut oil-fed animals survived to 20 mo. At this time the animals were killed to terminate the study.

Anti-dsDNA antibody. The results obtained for anti-dsDNA antibodies in the various groups of animals are presented in Fig. 3. The safflower oil-fed and untreated animals began developing anti-dsDNA antibodies at 4 mo of age. All animals in the safflower oil-fed group had positive anti-dsDNA tests by 8 mo of age. All untreated control animals were positive for anti-dsDNA antibodies at age 9 mo. Anti-dsDNA antibodies were not found in the PGE_1-treated animals until 8 mo of age; at 12 mo anti-dsDNA antibodies were found in all PGE_1-treated animals. Anti-dsDNA antibodies were not detected in any of the coconut oil-fed animals until 11 mo of age and only 57% of these animals had detectable anti-dsDNA antibodies at age 15 mo.

Thus, PGE_1-treatment caused a delay in appearance of anti-dsDNA antibodies from 4 to 8 mo of age when compared with untreated and safflower oil-fed animals. Strikingly, the formation of anti-dsDNA antibodies in coconut oil-fed mice was delayed until age 11 mo, no more than 57% were positive by age 15 mo, and 83% had anti-dsDNA antibodies at 18 mo of age. Thus, while safflower oil-fed, untreated, and PGE_1-treated animals achieved 100% positivity by age 12 mo, the coconut oil-fed animals never reached >83% positivity by 20 mo of age.

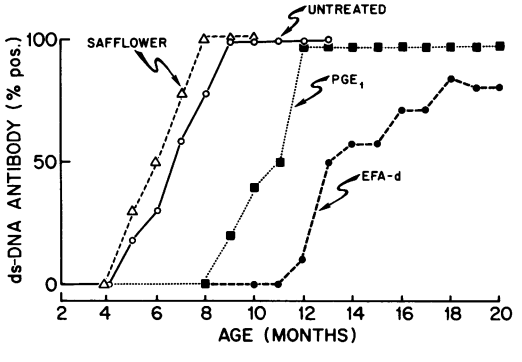


FIGURE 3 Serum ds-DNA antibody levels (% positivity) in all four groups of animals. EFA-d group vs. safflower oil-fed or untreated animals ($P < 0.05$). The PGE_1-treated group, although delayed as compared with safflower oil and untreated groups, was not statistically significant.

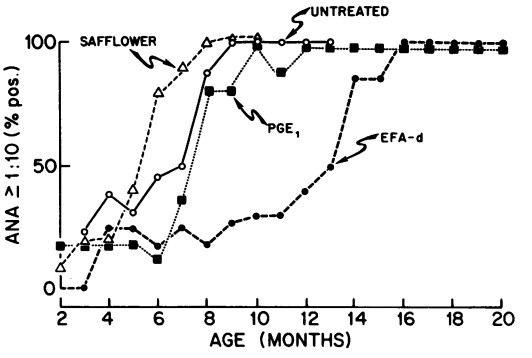


FIGURE 4 Serum antinuclear antibody levels in all four groups of animals. EFA-d group vs. safflower oil-fed or untreated animals ($P < 0.05$).

Antinuclear antibody. The results of the ANA tests for all four groups (positive > 1:20) are presented in Fig. 4. The time of appearance of ANA was similar in all groups. By age 9 mo, 100% of the safflower oil-fed animals and 98% of the untreated controls had positive ANA tests and 80% of the PGE_1-treated group were positive. However, only 25% of coconut oil-fed animals had positive tests at this time. Furthermore, the appearance of ANA in these animals continued to lag behind that found in the other three groups ($P < 0.05$). Whereas the PGE_1-treated animals had reached 100% positivity by age 12 mo, only 40% of coconut oil-fed animals were positive at the same age. The coconut oil-fed animals did not achieve 100% positivity until 16 mo of age.

The actual reciprocal ANA titers at 4, 6, 8, and 10 mo of age in all four groups are presented in Fig. 5. At 4 mo of age, titers were similar in all four groups of animals.

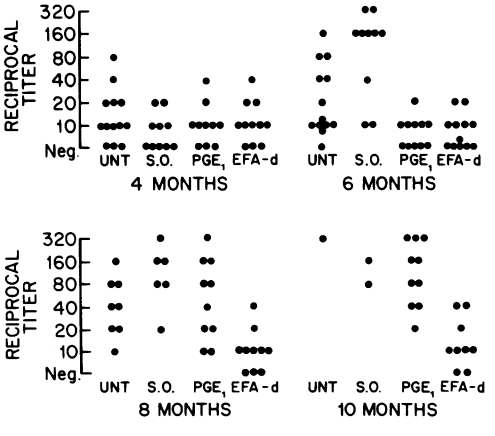


FIGURE 5 Actual reciprocal antinuclear antibody titers in all four groups of animals. Unt, untreated; S.O., safflower oil fed.

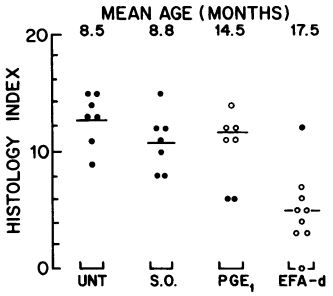


FIGURE 6 Renal pathology in all four groups of animals. The open circles represent those animals that were killed at the termination of the study, whereas the animals represented by the closed circles were sacrificed when the animals were dying. The mean age of the animals at the time tissues were obtained for determination of histology index is shown at the top of each group. The horizontal line in each group denotes the mean histology index. EFA-d vs. untreated ($P < 0.002$); EFA-d vs. safflower oil-fed ($P < 0.005$); EFA-d vs. PGE_1-treated ($P < 0.013$). There were no significant differences in other comparisons.

At 6 mo of age, 5 of the 13 untreated animals and 8 of the 10 safflower oil-fed animals had titers > 1:20. None of the PGE_1-treated or coconut oil-fed animals had titers > 1:20. At 8 mo, 5 of 8 untreated, 5 of 6 safflower oil-fed, and 6 of 10 PGE_1-treated animals had ANA titers > 1:20. In contrast, only 1 of 10 coconut oil-fed animals had a titer > 1:20.

By age 10 mo, only three animals were still alive in the untreated and safflower oil-fed groups and these animals had titers of 1:320, 1:160, and 1:80, respectively (see Fig. 5). Also at this time, 9 of the 10 PGE_1-treated animals had titers > 1:20. However, only two of the nine coconut oil-fed animals had titers > 1:20 (both animals had titers of 1:40).

Kidney histology. The effects of the various regimens on kidney histology are shown in Fig. 6. Seven animals in the untreated and safflower oil-fed groups were sacrificed when dying. The remaining animals in these groups died but tissues could not be obtained. In the PGE_1-treated group, two animals were sacrificed when moribund and three apparently healthy animals were killed at 13 mo of age. Two animals that were apparently healthy at 20 mo were killed to terminate the study. The coconut oil-fed group included one animal that died spontaneously, and three animals that were killed at 13 mo to compare with the PGE_1-treated animals that were killed at the same time. Five remaining animals survived to 20 mo. Although they appeared healthy, these animals were killed at 20 mo of age. The remaining animals, i.e., those from which tissue was not obtained, either died at a time of night when tissue could not be obtained or, in the case of some of the PGE_1-treated animals, died immediately following injection. Only the animals that died spontaneously were included in the survival data.

Separate scores were given for overall cellularity, amount of mesangial matrix, size of epithelial crescents, amount of interstitial infiltrate, fibrinoid change, and wire looplike alterations (Table II). These scores were then added together to give a composite score. Percentage of hyalinization was also assessed. The overall score for the untreated group was 13; for the safflower oil-fed group it was 11. The overall score for the PGE_1-treated group was 11; for the coconut oil-fed animals it was only 6. Thus, the coconut oil-fed animals had considerably less GN than did the untreated, safflower oil-fed, or PGE_1-treated animals (Fig. 7). The kidneys of the coconut oil-fed animals, in particular, had much less mesangial matrix, epithelial crescents, fibrinoid deposition, and wire looplike alterations.

The findings in those animals of the above groups that were killed at 13 mo of age (three PGE_1-treated and three coconut oil-fed animals) are presented in

TABLE II
*Effect of PGE_1 Treatment and Safflower Oil and EFA-deficient Diets on Kidney Histology**

Group	Number of animals	Cellularity	Mesangial matrix	Epithelial crescents	Interstitial infiltrate	Fibrinoid and wire loops	Overall score	Hyalinization	(Range)
								%	
Untreated	7	2+	4+	1+	3+	3+	13	19	(5–50)
Safflower oil	7	2+	3+	2+	2+	2+	11	6	(0–25)
PGE_1‡	7	3+	3+	1+	2+	2+	11	7	(0–50)§
EFA-deficient‡	9	2+	1+	±¶	2+	±	6	1	(0–5)

* Animals sacrificed when dying except for five animals in the PGE_1 group and eight animals in the EFA-deficient group (see below and Table III). Tissue was not obtainable in several animals because the animals died during the night or on a weekend.

‡ Each group includes three animals that were killed at 13 mo of age although they were not dying. The numbers also include two PGE_1 and five EFA-deficient animals that were killed at 20 mo of age although they were not dying.

§ Only one animal had >0%, and that was 50%.

¶ ± indicates slight involvement.

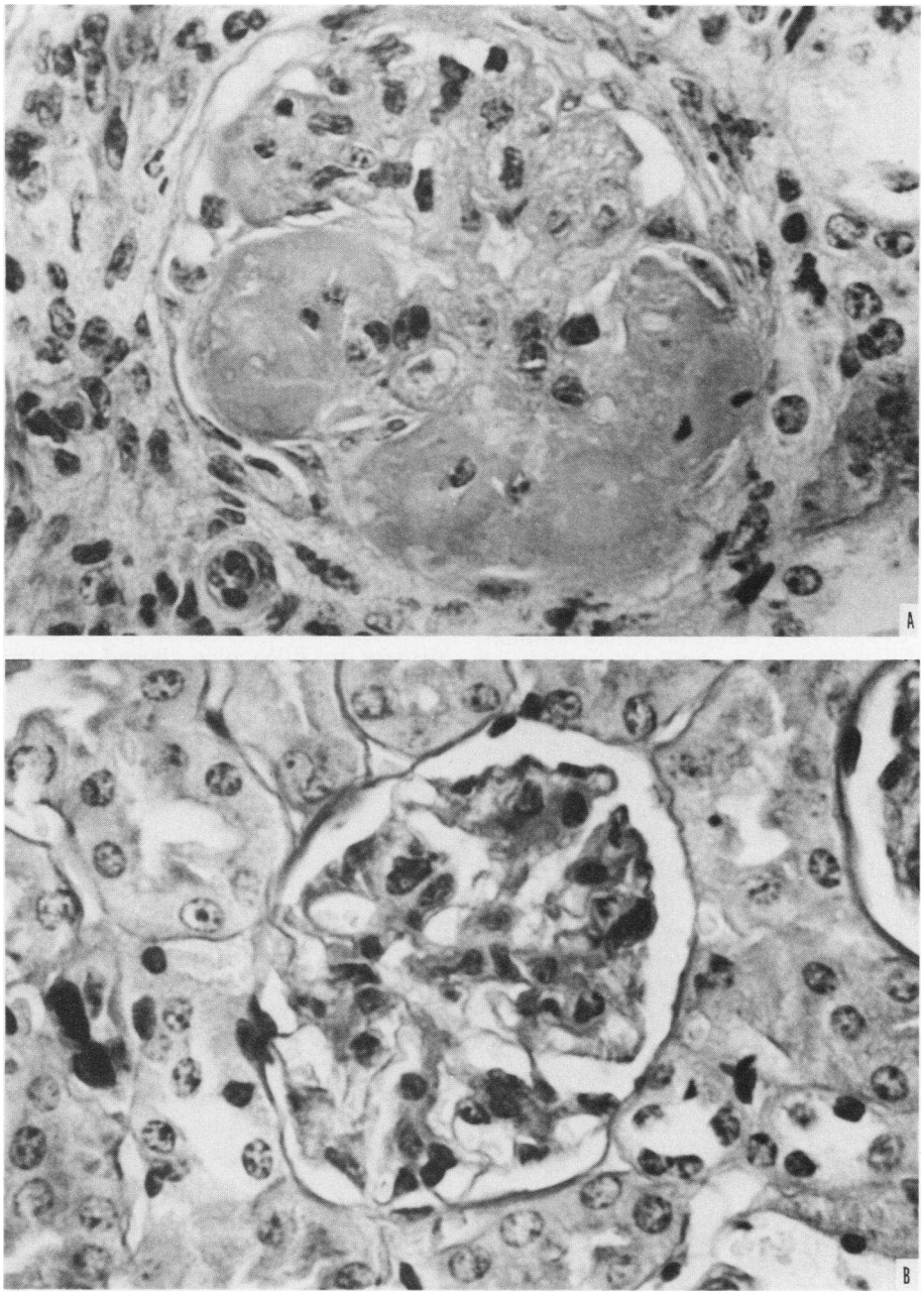


FIGURE 7 Kidney sections from untreated and EFA-deficient mice. (*a*) Glomerulus from an 8-mo-old untreated animal which demonstrates significant disease. Note the extensive involvement of capillary loops and mesangium, particularly in the lower half of the glomerulus. (*b*) Glomerulus from an EFA-deficient animal at 20 mo of age. There is moderate involvement (hematoxylin and eosin, ×240).

Table III. These mice were not moribund when they were killed, but were killed to evaluate the extent of renal involvement in these two apparently healthy groups of animals. The overall score in the three PGE_1-treated animals was 11. In marked contrast, the kidneys of the three coconut oil-fed animals had a score of only 3.5. It is interesting that neither the PGE_1-treated nor the coconut oil-fed animals had epithelial crescents (Table III).

Kidney immunofluorescence. The results of studies of kidney immunofluorescence are given in Fig. 8. Large IgG and IgM deposits were found in the un-

TABLE III

Effect of PGE_1 Treatment and EFA-deficient Diet on Kidney Histology in Animals Sacrificed at 13 Mo of Age

Group	Number of animals*	Cellularity	Mesangial matrix	Epithelial crescents	Interstitial infiltrate	Fibrinoid and wire loops	Overall score	Hyalinization	(Range)
								%	
PGE_1	3	3+	3+	0	3+	2+	11	13	0–50‡
EFA-deficient	3	1+	± §	0	2+	0	3.5	0	

* Average of three animals in each group killed at 13 mo of age.

‡ Only one animal had hyalinization > 0%, and that was 50%.

§ ± indicates slight involvement.

treated group, both in capillary loops and in the mesangium. The deposits in the safflower oil-fed group of animals were not quite as severe. There was less immunoglobulin deposition in PGE_1-treated and coconut oil-fed animals. This was also true in the PGE_1-treated and EFA-deficient animals that were killed at 13 mo of age (Table IV). Death occurred much later in the PGE_1-treated and EFA-deficient animals. Despite the fact that the EFA-deficient animals examined were much older than the untreated and safflower oil-fed animals, the glomerular fluorescent scores of their kidneys were somewhat lower.

In the majority of animals, both IgG and IgM were present. The kidneys of a few animals had either IgG or IgM. It is interesting that the kidneys of the three coconut oil-fed animals that were killed at 13 mo had only IgM present in the mesangium although in the capillary loops both IgG and IgM were present. Examples of fluorescent glomerular staining for IgG in untreated and EFA-deficient animals are shown in Fig. 9.

Vaginal smears. Vaginal smears prepared from 18-mo-old PGE_1-treated and EFA-deficient animals demonstrated cycling, when compared with CBA/J animals at the same age.

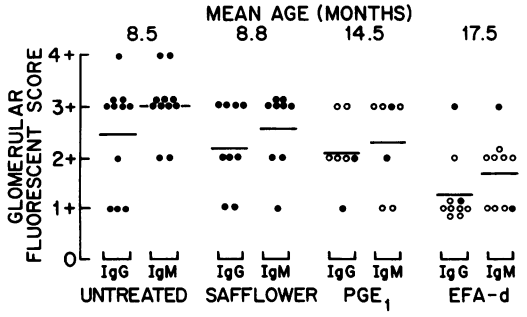


FIGURE 8 Glomerular IgG and IgM staining in all four groups of animals. For IgG values, EFA-d vs. untreated ($P < 0.02$); EFA-d vs. safflower oil-fed ($P < 0.03$); EFA-d vs PGE_1 ($P < 0.03$). For IgM values, EFA-d vs. untreated ($P < 0.02$); EFA-d vs. safflower oil-fed ($P < 0.03$); EFA-d vs. PGE_1 (NS). No other comparisons showed significant differences.

DISCUSSION

We undertook this study because of the finding of Zurier and co-workers (6, 7) that PGE_1 treatment had a beneficial effect on murine lupus erythematosus disease in B/W mice. Based on their findings we elected to investigate the effect of providing prostaglandin precursors in the form of a safflower oil-enriched diet on the course of the disease. We expected that such feeding might give a similar beneficial effect. However, the animals that were fed this diet had a survival similar to untreated control animals. Specifically, safflower oil-fed animals developed similar ANA and anti-dsDNA titers and similar degrees of renal involvement. In the course of these experiments, however, the following unexpected observations were made. Feeding B/W mice an EFA-deficient diet (*a*) significantly prolonged survival (to 20 mo in 55% of animals), (*b*) markedly diminished severity of their GN, (*c*) diminished amounts of IgG and IgM deposition in the renal glomeruli, and (*d*) significantly decreased the incidence and titer of anti-dsDNA and ANA.

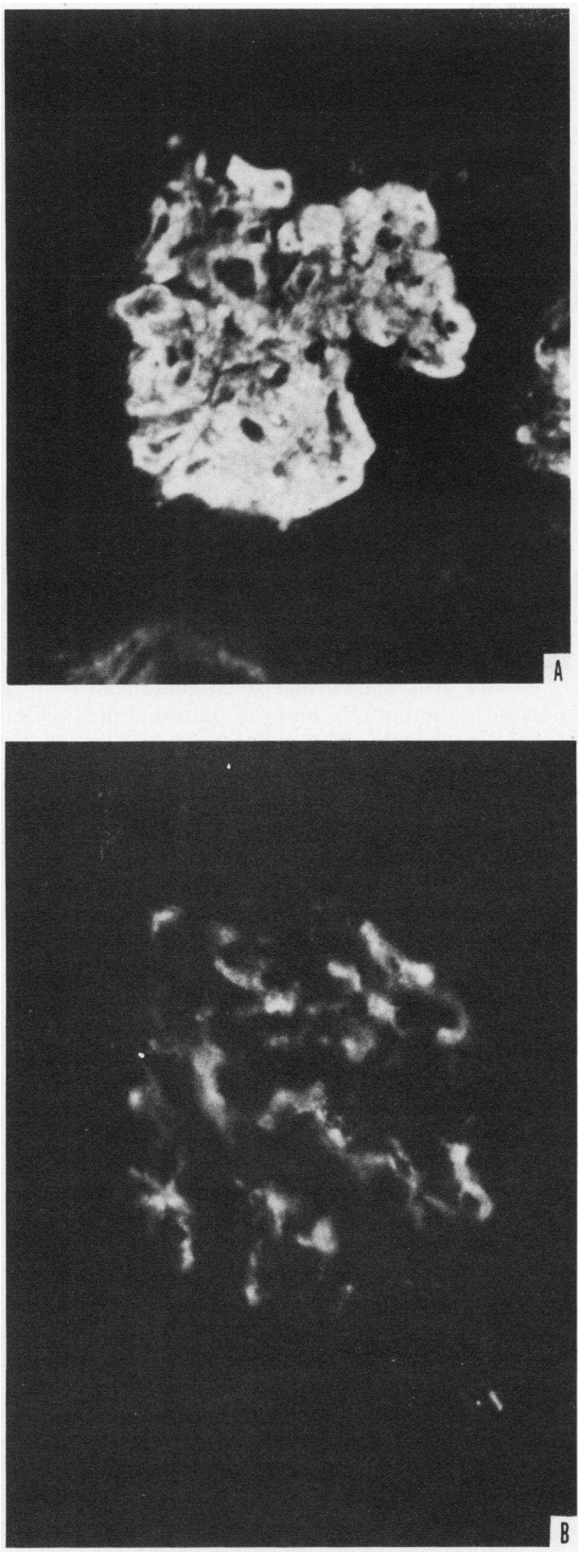
The animals treated with PGE_1 developed disease of intermediate severity. Several parameters of the disease process were improved in PGE_1-treated animals when compared with those of untreated and

TABLE IV

Effect of PGE_1 Treatment and EFA-deficient Diet on Kidney Immunofluorescence in Animals Sacrificed at 13 Mo of Age

Group	Number of animals	Capillary loop		Mesangium		Overall	
		IgG	IgM	IgG	IgM	IgG	IgM
PGE_1	3	2+	2+	1+	3+	2+	3+
EFA-deficient	3	1+	1+	±*	1+	±	2+

* ± indicates trace of staining.



safflower oil-fed mice. However, the EFA-deficient animals survived longer, had less GN, and lower ANA and anti-dsDNA levels than did PGE_1-treated animals.

Zurier and co-workers (6, 7) found that PGE_1-treatment of B/W mice decreased the severity of their lupus. Using PGE_1 in a dose of 200 μg injected subcutaneously either once or twice daily from 6 to 52 wk of age, treated animals did not develop anemia, clinical nephritis, or death (6). These investigators demonstrated subsequently (7) that PGE_1 treatment prevented glomerular deposition of immunoglobulins and complement and prevented the development of proliferative GN. We confirmed these findings in the present study. However, in contrast to our findings, Zurier and co-workers did not find that PGE_1 treatment prevented development of antibodies to ds-DNA. In their studies, the Farr assay as described by Luciano and Rothfield (13) was used and in our study the *C. luciliae* technique was used. Thus the difference in results may be due to differences in technique. Although the mechanism of action of the beneficial effect of PGE_1 on B/W disease is unknown, Zurier and co-workers (6, 7) have discussed various possibilities. These include preservation of T cell function and inhibition of platelet aggregation with concomitant suppression of release of mediators of inflammation from platelets and leukocytes. The latter effect would suppress deposition of immune complexes in the kidney glomerulus.

A number of studies have been conducted in which beneficial effects of various types of diets on murine lupus disease (of B/W mice) have been demonstrated. In studies by Fernandes and co workers (14, 15), it was shown that reduction of dietary fat decreased reproductive success but prolonged life and decreased propensity to autoimmunity in New Zealand Black mice. These investigators also showed that longevity of B/W and DBA/2f mice is prolonged dramatically by dietary restriction (16, 17). Restriction of caloric intake prolonged the life of B/W mice more than did protein restriction. It also has been shown that a low phenylalanine and low tyrosine diet prolonged life and decreased the severity of nephropathy in these animals (18). These results were believed not to be due to caloric restriction alone. Also it has been demonstrated in nonautoimmune strains of animals that a diet enriched in salts and vitamins, but limited in calories and/or protein, so-called underfeeding, slows the rate of aging and prolongs life (19). We do not believe that

FIGURE 9 Fluorescent staining for IgG in a glomerulus from (*a*) an 8-mo-old untreated animal which demonstrates significant staining and (*b*) a 20-mo-old EFA-deficient animal. Thus, although this animal is over twice the age of the untreated animal, it demonstrates much less staining for IgG (×240).

underfeeding played a significant role in the beneficial effects observed in the EFA-deficient animals of this study, since mean weights were similar in all four groups. Thus the beneficial effects in the coconut oil-fed animals do not appear to have been caused by reduced caloric intake.

A number of studies have also been conducted in which beneficial effects of EFA-deficient diets have been demonstrated in various animal models of immunologic disease. In studies by Denko (20), adjuvant arthritis was suppressed in rats deficient in EFA. The reduction in the chronic phase of adjuvant inflammation was restored to usual levels by feeding a small supplement of corn oil as a dietary source of EFA. It was concluded that the EFA deficiency diminished the adjuvant-induced inflammation by reducing available prostaglandins in the mediation of inflammation. Using various animal models of chronic inflammation, Bonta and co-workers (21–23) found a reduction in inflammation in EFA-deficient rats. They showed that carrageenin-induced hind paw inflammation is suppressed partially in EFA-deficient rats and that arachidonic acid given to these animals restored the suppressed carrageenin inflammation (22). Using kaolin-induced pouch granulomas, these same investigators demonstrated a reduction of exudate production in EFA-deficient rats when compared with normal animals (23). The exudates from normal rats contained large amounts of PGE but in the exudates from EFA-deficient rats the amount of PGE was reduced markedly. In studies by Mertin and Hunt (24), mice fed a diet deficient in polyunsaturated fatty acids showed a relative immunopotentiation, as indicated by accelerated skin-allograft rejection and decreased incidence and rate of development of methylcholanthrene-induced tumors. Their cell-mediated immune responses appeared to be potentiated. Enhancement of delayed hypersensitivity to purified protein derivative in rats fed an EFA-deficient diet has also been reported by Parnham and co-workers (25). It is of interest that similar effects were obtained with PGE_1 treatment.

The design of the present study was not directed toward the elucidation of the biochemical basis for the observed beneficial effects of EFA-deficient diet on lupus. It is possible that a relative EFA deficiency is beneficial if an arachidonic acid metabolite(s) is involved in mediating murine lupus in the B/W animals. Many of the arachidonic acid metabolites from either the lipoxygenase pathway (12-hydroxy-eicosatetraenoic acid, 12-hydroperoxy-eicosatetraenoic acid, leukotrienes, etc.) or cyclo-oxygenase pathway (prostacyclins, thromboxanes, prostaglandins, etc) could be involved in mediating murine lupus by virtue of their biological activity in chemotaxis, vascular permeability, and other factors involved in inflammation (22, 26–30). For example, in a very recent study by Cook and co-workers (31, 32) it was demonstrated that EFA-deficient diets were beneficial in rats with endotoxic shock. This effect was believed to result from the decreased production of thromboxane A_2.

Because the EFA-deficient diet can impair fertility (33–35), it is possible that this could also influence the disease process in view of the correlation of B/W disease severity with estrogen availability and suppression of disease severity by androgen treatment in these mice (36, 37). The EFA-deficient diet might reduce the rate of estrogen production in the female mouse with resultant improvement. Such a mechanism does not seem likely, however, since we found that the EFA-deficient and PGE_1-treated animals were having regular estrus cycles.

An additional consideration in explaining the improvement in the coconut oil-fed animals is the fact that coconut oil is rich in lauric and myristic acids. These saturated fatty acids have been shown to act as inhibitors of the conversion of arachidonic acid to PGE_2 (38). Thus, it is possible that these fatty acids might have a direct inhibitory effect on arachidonic acid metabolism to the extent that this might be going on in animals on an EFA-deficient diet.

Finally, it is known that arachidonic acid is incorporated preferentially into the phospholipids of activated lymphocytes (39) and constitutes almost 20% of the total phospholipid fatty acid content of macrophages (40). Thus, a lack of EFA might conceivably impair lymphocyte and/or macrophage function with a resultant beneficial effect in B/W mice.

Irrespective of the exact mechanism, the feeding of an EFA-deficient diet produced a profound beneficial effect in animals with murine lupus erythematosus. Our results suggest that the availability of EFA is a factor in the evolution of the disease process in these mice. Studies are now in progress to further elucidate the biochemical basis for the beneficial effects of an EFA-deficient diet on the development of murine lupus erythematosus.

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