Influence of diet on survival of mice

(nutrition/autoimmunity/longevity/calories/protein)

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ABSTRACT   The longevity of mice of the (NZB ×
NZW)F₁ (B/W) strain and the DBA/2f strain of mice is dra-
matically prolonged by dietary restriction. B/W mice are sus-
ceptible to, and die at an early age from, immunocomplex
nephritis. Mice of the DBA/2f strain are also relatively short-
lived. Restriction of caloric intake prolonged life of B/W
mice more than did protein restriction. DBA/2f mice showed
prolongation of life when the diet was restricted only with
respect to protein. Calorie restriction alone prolonged life
less in DBA/2f mice than in B/W mice. These observations
show that dietary manipulations have profound effects on
immunity functions, including inhibition of the development
of life-shortening autoimmune disease.

Interest in the effect of diet on immunity, malignancy and
longevity has been expressed through the years (1–15).
McCay et al. (16) showed that placing weanling rats on a
diet restricted in calories promotes longevity. Walford et al.
(17) showed in mice that calorie restriction during growth
delays development of full immunologic vigor but prolongs
maintenance of immunologic competence. Aref et al. (18)
showed in humans that protein-calorie deprivation early in
life causes increased susceptibility to infection and gross de-
ficiencies of cellular and humoral immunity. Smythe et al.
(19) and Edelman et al. (20) found that in man protein and
protein/calorie deprivation leads to deficiencies of cellular
immunity attributable to deficiency in function of thymus-
dependent (T)-lymphocytes.

Jose et al. (21) observed that although malnourished Aus-
tralian aboriginal children frequently exhibited rheumatic
heart disease, they rarely showed symptoms attributed in
well-nourished populations to post-streptococcal rheumatic
fever. Jose et al. (22) showed that antibody responses of
these nutritionally deprived aboriginal children to certain
antigens can be grossly deficient, whereas in vitro responses
of T-lymphocytes to plant lectins are better preserved. Be-
cause analysis of the relationship of immunologic perturba-
tions to the nutritional deficiency may be difficult under
field conditions, we launched an analysis of the influence of
nutrition on immune functions of experimental animals
under controlled laboratory conditions. Jose et al. (23–26),
Cooper et al. (27), Good et al. (28, 29), and Kramer and
Good (30) showed that in mice, rats, and guinea pigs moder-
ate chronic dietary protein restriction depresses antibody
production while increasing or permitting maintenance of
certain cell-mediated immunities. Extreme protein depriva-
tion produced deficiency of both cellular and humoral im-
munity. Profound nutritional deficiencies exerted influences
from which the animals were slow to recover. In an earlier
study with NZB mice we observed that breeding capacity is
deficient (31). Comparisons were made, using two commer-
cial diets that differed in proportion of animal fat and pro-

tein. Reduction of dietary fat decreased reproductive success
but prolonged life and decreased propensity to autoimmun-
ity in NZB mice (32, 33). A well-defined diet low in protein
resulted in maintenance of cell-mediated and humoral im-
munity functions which usually decline with age in NZB
mice (34–36). Autoimmune hemolytic anemia was slower to
develop but was not prevented, nor was longevity increased.
These studies dissociated to some degree changes in immu-
noglobulin levels and the declining vigor of cell-mediated
immunity of NZB mice from expression of lethal autoim-
mune anemia. Dubois et al. (37) also noted that a low-phe-
nylalanine and low-tyrosine diet interfered with disease ex-
pression and prolonged the life of (NZB × NZW)F₁ mice.

The present investigation concerns influence of different
diets on longevity of short-lived, autoimmunity-susceptible
mice. (NZB × NZW)F₁ (B/W) mice were studied because
this hybrid is short-lived due to the development of autoim-
mune renal disease early in life (38, 39). In most environ-
ments these animals die between 8 and 15 months of age
and rarely survive beyond 450 days. Death occurs earlier in
female than in male mice and is attributable to renal injury
due to antigen–antibody complexes (40). The present study
shows that prolongation of life occurred in B/W mice when
calories were restricted. In DBA/2 mice, reduction of pro-
tein intake was associated with increased longevity.

MATERIALS AND METHODS

Animals. Inbred, 3-week old DBA/2f and (NZB ×
NZW)F₁ (B/W) hybrid mice raised at the University of
Minnesota mouse colony were used for the present study.
Four mice of the same sex were kept in each cage, weighed
weekly, and examined daily; all deaths were recorded. The
mice were otherwise maintained under standard conditions
of relative humidity and temperature [55 ± 2% and 72 ±
2°F (22 ± 1°C), respectively]. Twelve hours of light and 12
hr of darkness were provided automatically each day.

Diets. Composition of diets is summarized in Tables 1 and
2. Normal and low protein levels chosen were based on stud-
ies of Jose et al. (23). Diets were prepared from ingredients
(casein, salt, vitamin mixture) purchased from Nutritional
Biochemicals, Cleveland, Ohio, and the remaining ingredi-
ents were each obtained from a single source (see Table 1).
All the diets were prepared weekly and stored at 4°C until
used. A fixed amount of food equal to 5 g of dry weight per
adult mouse was given once each day between 8 and 10 a.m.
to groups on normal calorie intake, and exactly half that
amount (2.5 g) was provided to the groups on restricted calo-
ric intake. However, the calorie-restricted diets contained
twice the concentration of salts and vitamins; otherwise the
rest of the ingredients remained at the same levels and thus
were given in one-half the amount provided for the control
mice. The amount of food given in the immediate post-

Table 1. Composition of diets used for study of longevity in B/W and DBA/2f mice

| Ingredients* | Diets | | | |
	I	II	III	IV
Casein[a]	220	60	220	60
Dextrose[b]	330	410	255	335
Cornstarch[c]	330	410	255	335
Corn oil[d]	50	50	200	200
Salt mixture[e]	40	40	40	40
Vitamin mixture[f]	20	20	20	20
Agar[g]	10	10	10	10
Water	1000	1000	1000	1000
Approximate calories/1000 g	3970	3970	4720	4720

* Numbers are grams of ingredients. Agar was dissolved first in boiling water and other ingredients were then added and mixed well with a blender, except for the vitamin mixture, which was incorporated only after the mixture had cooled. Calorie restricted diet group received salt and vitamin mixture equal to that of normal calorie group.

[a,e,f] From Nutritional Biochemicals, Cleveland, Ohio. [b] From Mallinckrodt Chemicals, St. Louis, Mo. [c] From North American Food Service Corp., Chicago, Ill. [d] From Swift Edible Co., Chicago, Ill. [g] From Difco Laboratories, Detroit, Mich.

weaning period at 3–8 weeks was slightly lower than that indicated above and was gradually increased up to 5 g at 8 weeks. Thereafter, the amount of food for each mouse remained constant from 8 weeks throughout the remainder of the life span. Care was taken to present the feeding within the cages in a special distribution permitting each mouse to consume his full share of the diet each day. In general, mice on the restricted diets were similar to one another in size and vigor.

RESULTS

On Fig. 1, the growth curves of B/W mice on diets of different composition are compared. It will be seen from the figure that growth is moderately retarded: (1) on the low fat, low calorie diet; (2) on the low protein diet; (3) on the low protein, low calorie diet; (4) on the low protein, high fat diet; and (5) on the low protein, low calorie, high fat diet— as compared to that of mice on a diet composed of 22% protein, 5% fat, and 20 calories per day. Similar influences on growth were observed in DBA/2f mice, but the influence of the restricted diets was somewhat more impressive in B/W mice. In Figs. 2 and 3, the survival times of B/W mice fed diets of various compositions are compared. Dietary intake of protein, fat, carbohydrate, and total calories were manipulated. It will be seen from Table 3 that all of the animals fed the control diet of 22% protein, 5% fat, 20 calories per day had died by 465 days of age, and the mean times of

Table 2. Protein and fat composition of diets, and calories/day per mouse

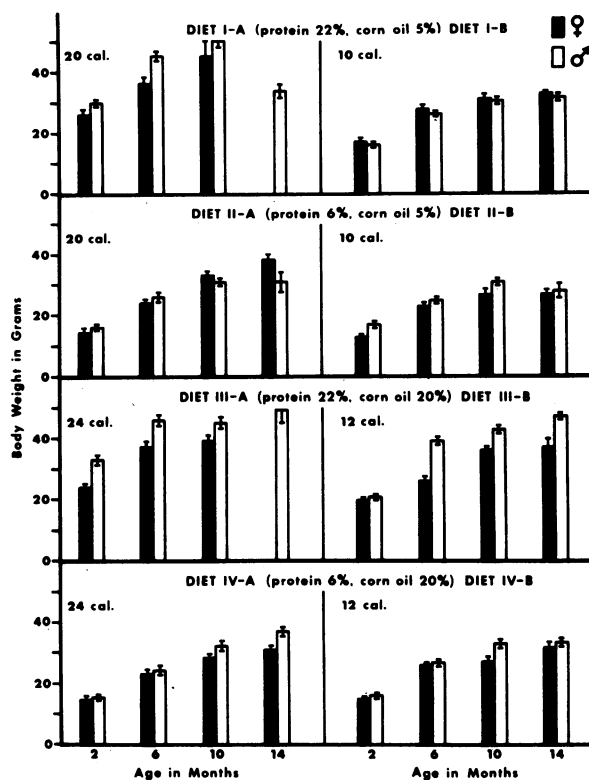| | Diets | | | | | | | |
	I-A	I-B	II-A	II-B	III-A	III-B	IV-A	IV-B
Protein %	22	22	6	6	22	22	6	6
Corn oil %	5	5	5	5	20	20	20	20
Calories	20	10	20	10	24	12	24	12

FIG. 1. Growth of B/W mice on diets of different composition.

death (± SEM) for females and males were 334 ± 18 and 371 ± 13 days, respectively. The median survival time, 20% survival time, and longest survival times are also recorded in Table 3. Animals fed low protein diet with half the caloric intake lived significantly longer than did mice fed the normal calorie diet. A similar influence in prolonging survival
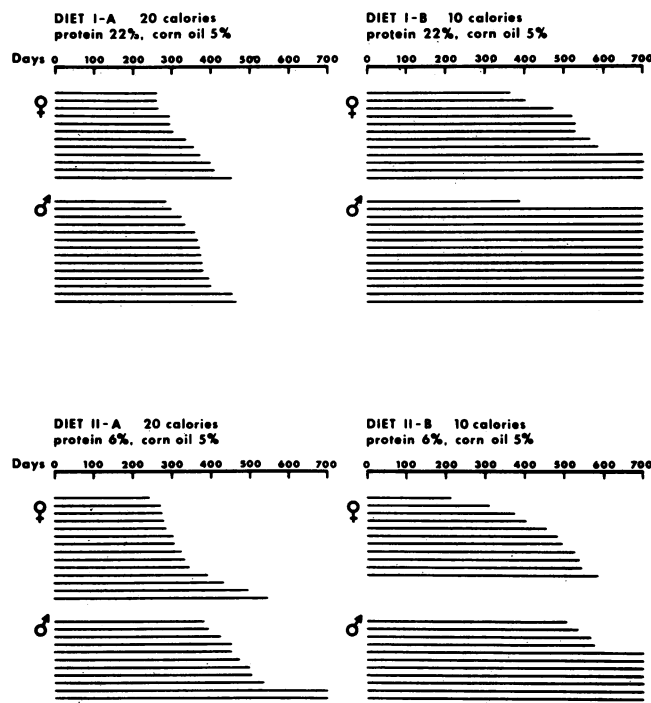

FIG. 2. Survival of B/W mice on diets of different composition; each line represents life span of a single mouse.

Table 3.   Influence of diet on number of days for survival of mice

B/W Strain

	Diet I-A, 20 cal/d		Diet I-B, 10 cal/d		Diet II-A, 20 cal/d		Diet II-B, 10 cal/d	
	Females	Males	Females	Males	Females	Males	Females	Males
Mean ± SEM	334 ± 18	371 ± 13	568 ± 34*	674 ± 25*	347 ± 24	498 ± 25	447 ± 34	649 ± 22*
Median	317	350	550	700†	306	487	481	700†
20% survival	400	400	700†	700†	433	600	545	700†
Longest survival	452	450	700†	700†	545	650	585	700†

	Diet III-A, 24 cal/d		Diet III-B, 12 cal/d		Diet IV-A, 24 cal/d		Diet IV-B, 12 cal/d	
	Females	Males	Females	Males	Females	Males	Females	Males
Mean ± SEM	354 ± 25	447 ± 23	492 ± 35*	582 ± 33*	356 ± 28	435 ± 21	561 ± 34*	638 ± 23*
Median	318	440	467	557	331	488	547	700†
20% survival	460	549	585	700†	355	519	700†	700†
Longest survival	565	610	700†	700†	585	600	700†	700†

DBA/2f Strain

	Diet I-A, 20 cal/d		Diet I-B, 10 cal/d		Diet II-A, 20 cal/d		Diet II-B, 10 cal/d	
	Females	Males	Females	Males	Females	Males	Females	Males
Mean ± SEM	434 ± 53*	420 ± 33	414 ± 26	357 ± 40	525 ± 46*	625 ± 46*	429 ± 46	525 ± 29*
Median	435	465	430	418	523	650†	497	478
20% survival	650†	524	512	444	650†	650†	550	650†
Longest survival	650†	560	560	498	650†	650†	600	650†

d = day.
* Calculated on basis of 700 day survival for animals alive at 700 days (650 days for DBA/2f).
† Mice still surviving beyond 700 days (650 for DBA/2f).

was seen in the group fed a diet low in calories but with a higher proportion of protein and fat (corn oil). Here again, those given one half of the usual calories survived much longer. The most dramatically beneficial influence of diet on longevity of this relatively short-lived hybrid mouse was

FIG. 3.   Survival of B/W mice on diets of different composition; each line represents life span of a single mouse.

exerted by caloric restriction, regardless of the relation of protein to fat content in the diet. Among the mice fed 22% protein, 5% fat, low calorie diets, 70% survived beyond 700 days, whereas none of the mice given 22% protein, 5% fat, and 20 calories per day lived beyond 500 days. The 20% survival time and longest survival time have yet to be determined for the mice on the low caloric intake, since more than half of these mice are still living beyond 700 days.

The results of dietary manipulation in mice of DBA/2f strain were also of interest and were somewhat different from those obtained in B/W mice. As will be seen in Fig. 4 and Table 3, DBA/2f female and male mice fed the standard diet had mean survival times of 434 ± 53 and 420 ± 33 days, respectively. The longest survivors and the 20% survivors (females) lived to more than 650 days. Giving DBA/2f mice half the amount of calories decreased the longest life span of males to under 500 days but did not change significantly the mean survival time. A low protein, normal calorie intake, on the other hand, significantly increased the proportion of mice experiencing prolonged survival. In this strain, no advantage was found to result from lowering the calories in addition to lowering the protein intake, and calorie restriction *per se* did not favor prolonged survival. The DBA/2f mice given low calories seemed to be more susceptible to infection, e.g., chronic pneumonia, than were mice taking a diet higher in calories, under the conditions of these experiments. Higher protein and calorie intake appears to have an adverse effect on survival of DBA/2f mice, whereas lower protein intake contributes to prolongation of life.

DISCUSSION

Extensive earlier studies show that one can manipulate nutritionally numerous processes vital to survival of mice, rats,
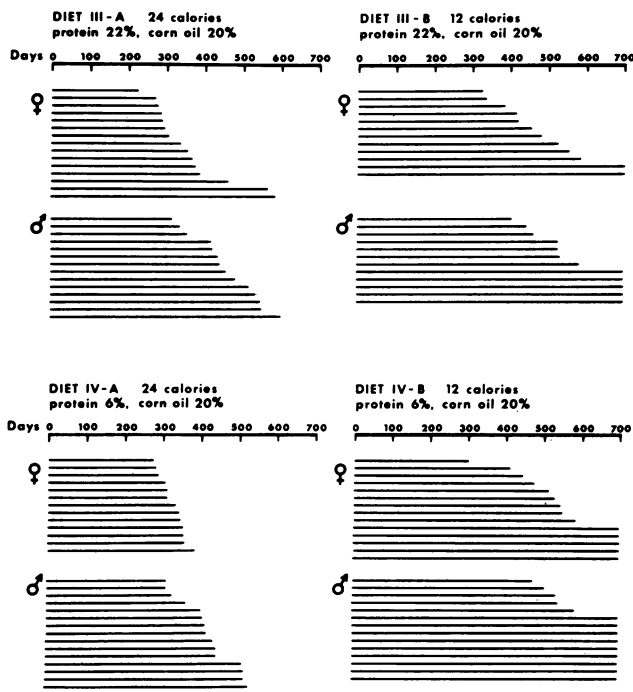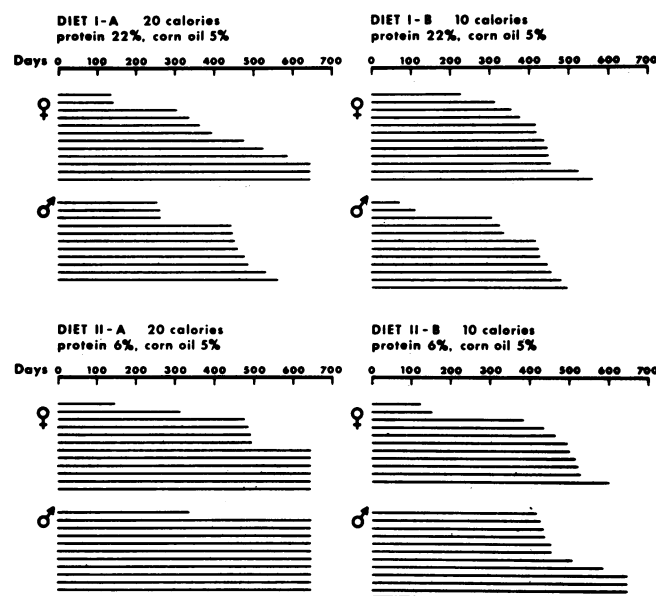
FIG. 4. Survival of DBA/2f mice on diets of different composition; each line represents life span of a single mouse.

guinea pigs, and man (29). Among these are processes involved in aging (16, 17), propensity to develop tumors or malignancies (11–15), resistance to infection (5, 18), function of cellular systems responsible for immunity, and functions of the biologic amplification systems and effector processes of immunity (17, 23, 25, 28). In the study presented here, we report that length of life in the short-lived, autoimmunity-susceptible B/W strain of mice can be markedly increased if the mice have been given diets of various compositions that are low in calories. Little influence on survival of these mice could be attributed to restriction of protein or manipulation of fat composition in the diet. When calorie intake was not altered, similar but somewhat less dramatic influences of diet on longevity were observed in DBA/2f mice. Dietary protein restriction prolonged life of DBA/2f mice. Thus, although diet was shown to have a profound influence on longevity in mice of both strains, the influence of specific dietary components was clearly modified by genetic factors and/or by the propensity to develop certain diseases in these two strains.

Of particular importance in the present study was the finding that survival of B/W mice can be more than doubled by calorie restriction. This influence of diet on survival was greatest in males but clearly present also in females of this strain. Extensive further experimental analyses will be required to elucidate the mechanism of this extraordinary influence. At least three viruses have been linked by different investigators to autoimmune disease and development of malignancy in NZB mice (41–43). The most intimate link seems to be to the xenotrophic virus of Levy (42). Certainly, the influence of diet on production of virus or viruses will have to be analyzed. It seems most likely to us that at least part of the dietary influence will be found to be attributable either directly or indirectly to the nutritional influence on immunological processes. Already, dietary influences on immunity have been shown to be profound. It seems likely that the influence of diet has altered the immunologic injury responsible for the rapidly progressive, highly destructive renal disease that occurs in the B/W strain, which has clearly been implicated as a cause of early death in these animals. An important influence on this lethal disease must have oc-

curred, since these hybrids have lived so long. Whether the immediate process being altered by the nutritional manipulation in this model is the lymphoid cells in general, helper or suppressor T-cell population, or processes involved in handling antigen–antibody complexes, or even processes underlying the production of virus (44), must be determined. However, the fact that a similar though less dramatic influence is exerted by protein rather than calorie restriction on DBA/2f mice suggests that a fundamental process associated with longevity in the two strains may have been influenced by dietary manipulation. Certainly, the influence of dietary restriction on tumor development in B/W mice must also be studied. NZB mice die of autoimmunity, infection, or malignancy. B/W mice usually die of kidney damage at an early age so that the virus which seems to have the capacity to produce malignancy might not express its oncogenic potential unless the life span of these animals is prolonged. Thus, the oncogenic influence of a chronic virus infection to produce malignancy in B/W mice that may not be expressed in the host on a high protein, high calorie diet because of the shortness of life, might be expressed when the dietary restriction permits a much longer life. Alternatively, the dietary restriction might delay expression of the malignancy as well as of the autoimmunity. The latter seems to us most likely because the animals given the low caloric intake continue to live very long, and none have developed lymphomas. Methods are available which will permit quantitative analyses of virus production, antibodies against virus, virus neutralizing factors, circulating immune complexes, deposition of immune complexes in kidneys, development of renal and/or malignant disease, and numbers and functions of cells of lymphocyte subpopulations. The influence of diet on each of these processes must be systematically investigated.

It seems particularly important, in light of the findings reported here, to design further experiments to evaluate whether this powerful influence on longevity can be exercised after reproduction has taken place. Further experiments must also be done to evaluate whether or not the profound dietary influence can be exerted even after the onset of autoimmune disease. If we are to think of manipulating comparable human disease, e.g., lupus erythematosus, in light of these findings, we must know whether the diet which interferes with the destructive effect of autoimmune, lupus-like disease needs to be given prophylactically, or whether therapeutic nutritional intervention might be possible. It seems likely to us that principles derived from manipulation of life span of B/W mice and prevention of devastating disease in these animals might be the basis for prevention or manipulation of certain very destructive human diseases. Already, favorable indications that serious dermatitis and human renal disease can be favorably affected by nutritional manipulation has been forthcoming (45, 46). Indeed, Carmena and Shapiro (46) have found that chronic nutritional deprivation in patients with diffuse glomerulonephritis leads to improved kidney function and permits avoidance of therapeutic dialysis. It seems quite possible that, with further studies, nutritional manipulation may become important far beyond reducing urea load in treating certain immunologically based renal diseases of man. Whether the advantages of an otherwise properly constituted but still "calorie-deficient" diet will outweigh its disadvantages in human populations can only be determined by field studies. That further study of animal model systems like those employed here is necessary, is clearly indicated by the influence of diet

on survival of B/W mice and by the striking differences in the influence of dietary manipulation on longevity of mice in the two strains studied.

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