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SURVIVAL AND DISEASE PATTERNS IN C57BL/6J MICE SUBJECTED TO UNDERNUTRITION

K. E. CHENEY,* R. K. LIU, G. S. SMITH, R. E. LEUNG, M. R. MICKEY† and R. L. WALFORD

Departments of Pathology and Biomathematics,† University of California, Los Angeles, CA 90024, U.S.A.

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INTRODUCTION

THE EVENTUAL goal of aging research is to promote a longer, more disease-free lifespan. In this regard, the studies of McCay (McCay *et al.*, 1935) and Ross and Bras (Ross, 1961; Ross and Bras, 1965) remain classic. In fact, long-term undernutrition is thus far unchallenged as the sole method of retarding aging and extending lifespan in homeotherms (Sacher, 1977). This report describes one such attempt in long-lived mice, chosen for their suitability for immune functional studies, and for their superiority to short-lived strains as a gerontologic model. The increased maximum survival and lessened tumor incidence in the undernourished mice, as reported here, may relate to their better preservation of immune function into later life (Gerbase-DeLima *et al.*, 1975); however, further experiments will be necessary to adequately characterize the etiology of these phenomena.

METHODS AND PROCEDURE

Four cohorts of C57BL/6J mice were used, each composed of 2–4 dietary groups, including animals undernourished both pre- and/or post-weaning, together with appropriate controls (Table 1). Both males and females were present in cohort I, whereas cohorts II, III and IV were comprised of females only. All animals were singly caged postweaning. The postweaning experimental diets were based on those of Ross and Bras (Ross, 1961; Ross and Bras, 1973), which yielded the best survival in rats when fed in restricted amounts. Essentially, the normo-caloric diet consisted of 21.6% casein, 39.1% sucrose, 15.0% cornstarch, and 13.5% cottonseed oil (cohorts I, II and III) or corn oil (cohort IV), with the restricted diet being similar, except that salts and vitamins were doubled at the expense of cellulose (cohorts I, II and III) or cellulose and cornstarch (cohort IV), in order to preclude malnutrition. In addition, salts and choline were increased at the expense of cellulose (N/N) or cellulose and cornstarch (R/R) in cohort IV, vs cohorts I, II and III, in order to provide a better basal diet. Experimental diets for cohorts I, II and III (Table 2) were supplied by General Biochemicals in pellet form, and for cohort IV, by Nutritional Biological Co. in pellet form initially, and in powder form past the 80th week of age. The exact compositions of the diets of cohort IV were given previously (Gerbase-DeLima *et al.*, 1975). The N/N and R/N mice received 7 portions/week, and the N/R and R/R mice, 4 portions/week (Table 1). Restriction prior to weaning was accomplished in the R/N and R/R mice by assigning them to litters of 9 and/or by separating them from the mothers every other day after day 2 (cohort I) or day 7 (cohort IV) (Table 1). By contrast, the N/N and N/R mice were with the mothers continuously, with the N/N mice in cohort IV specifically being assigned to litters of five. Since early restriction retarded growth, weaning time was delayed slightly in the later cohorts (Table 1) to ensure better survival during the first month of life in the R/N, N/R and R/R groups. The Lab Chow groups were included to allow comparison with the conventional feeding mode, and thus were fed normally before weaning, and given Purina Lab Chow *ad libitum* thereafter.

Weighings were performed monthly on those animals held until natural death. At autopsy, these animals were examined grossly, and suspected abnormalities were studied microscopically. Tumor classifications, as defined here, were described previously (Smith *et al.*, 1973). Those animals too autolyzed for satisfactory examination were excluded from the disease analysis, but were included in the lifespan analysis. Unfortunately, those few animals dying past 2 yr of age in cohort II were not autopsied, due to an oversight. Cohort III was

*To whom reprint requests should be addressed.

TABLE 1. PRE- AND POSTWEANING DIETARY REGIMENS FOR GROUPS OF C57BL/6J MICE WITHIN FOUR COHORTS

Cohort	Symbol	Sex	Preweaning dietary regimen	Day of weaning	Postweaning dietary regimen*	Calories/ week postweaning
I	N/N	M&F	With mother	21	~15 cal. MTuWTh; ~45 cal. F	~105
	R/N	M&F	Separated from mother every other day after day 2	21	~15 cal. MTuWTh; ~45 cal. F	~105
	N/R	M&F	With mother	21	~15 MW; ~30 cal. F	~60
	R/R	M&F	Separated from mother every other day after day 2	21	~15 MW; ~30 cal. F	~60
II	Lab Chow	F	Received from Jackson Laboratory at approx. 4 weeks of age		Purina Laboratory Chow	*ad. lib.*
	N/N	F	Same as above		~15 cal. MTuWTh; ~45 cal. F	~105
	N/R	F	Same as above		~15 cal. MW; ~30 cal. F	~60
III	N/N	F	With mother	24	~15 cal. MTuWTh; ~45 cal. F	~105
	N/R	F	With mother	24	~15 cal. MW; ~30 cal. F	~60
IV	Lab Chow	F	With mother	28	Purina Laboratory Chow	*ad lib.*
	N/N	F	With mother, 5 to a litter	28	~15 cal. MTuWTh; ~45 cal. F	~105
	R/R	F	9 to a litter; separated from mother every other day after day 7	28	~15 cal. MW; ~30 cal. F	~60

*Portions prior to 2 months of age were somewhat smaller, but represented amount fully consumed by N/N mice.

TABLE 2. COMPOSITION OF POSTWEANING DIETS FOR COHORTS I, II AND III

	g dry weight/kg diet	
	N/N, R/N	N/R, R/R
Casein, vitamin-free test	216.00	216.00
Cornstarch	150.00	150.00
Sucrose	391.00	391.00
Hydrogenated cottonseed oil	135.00	135.00
Salt mix, USP XIV	40.00	80.00
Non-nutritive fiber (cellulose)	66.0663	24.1326
P-Aminobenzoic acid	0.01	0.02
Biotin	0.0003	0.0006
Vitamin B_{12} w/mannitol (0.1%)	0.015	0.030
Calcium pantothenate	0.02	0.04
Choline chloride	1.00	2.00
Folic acid	0.0055	0.0110
Inositol	0.400	0.800
Menadione	0.005	0.010
Niacin	0.02	0.04
Pyridoxine HCl	0.005	0.010
Riboflavin	0.010	0.020
Thiamine HCl	0.005	0.010
Dry vitamin A (500,000 units/g)	0.034	0.068
Dry vitamin D_3 (500,000 units/g)	0.0034	0.0068
Dry vitamin E acetate (250 units/g)	0.363	0.726
Zinc oxide (ZnO)	0.0750	0.375

excluded entirely from the disease report, due to incomplete autopsy data. Animals culled for experiments—described earlier (Walford *et al.*, 1972, 1973/74; Gerbase-DeLima *et al.*, 1975)—were also excluded from the disease analysis, and appropriate correction factors were applied in the lifespan analysis (Kaplan and Meier, 1958).

With the exception of the Gompertzian analysis, data analyses were performed largely using BMDP Biomedical Computer Programs, including BMD08V (Dixon, 1973), BMDP1L, BMDP3D and BMDP1F (Dixon and Brown, 1977). Survival curves were depicted by product-limit analysis (Kaplan and Meier, 1958; Dixon and Brown, 1977), using a Versatec 1200 printer/plotter. Product-limit analysis generates a point for every natural death, with correction factors being introduced for any accidental or sacrificial deaths, as mentioned above. Any deaths prior to 8 weeks of age were considered neonatal, and were excluded. Gompertzian parameters were estimated by the method of maximum likelihood (Garg *et al.*, 1970), and goodness-of-fit was tested using the Cramer-von Mises w^2 statistic (Mickey *et al.*, 1963). All parametric statistical tests were two-tailed, with separate variances for both samples being used exclusively in the calculation of two-sample *t*-statistics. Oftentimes, statistical comparisons were made for all possible combinations between two groups of the same sex within each cohort, without adjusting for multiple comparisons. While this may not be the best statistical procedure, it was taken to be the most satisfactory and simplest way to spot trends occurring across more than one cohort, since the parameters of the statistical test were always the same, i.e. only two groups were compared at once, despite the fact that the total number of groups differed among cohorts.

RESULTS

Survival curves are shown in Fig. 1, and relevant data are summarized in Table 3. The nonrestricted and less severely restricted groups, i.e. Lab Chow, N/N and R/N, generally had better survival during mid-life, and sometimes during early life, than the more restricted groups, i.e. N/R and R/R (Fig. 1). Later in life, the situation usually reversed, such that the more restricted groups had better long-lived survival. In cohort I, the R/N mice, particularly the males, appeared to have the greatest survival advantage throughout much of life, but this could not be confirmed in the other cohorts, where R/N mice were absent. Mean survival did not differ significantly, except in cohort I, where the N/N females had a greater mean lifespan than the N/N males ($p = 0.029$, as judged by the *t*-test) (Table 3).

Maximum survival was always greatest in the most severely restricted group, except in cohort I, where the R/R females had the poorest overall survival and the shortest maximum survival. According to a signed-rank test comparing the maximum survival of those groups restricted postweaning with those not restricted postweaning (i.e. N/N and N/R, R/N and R/R, but in cohort IV, N/N and R/R), a value of $p = 0.04$ was obtained, indicating that postweaning restriction significantly extended maximum survival. In cohort I, because the R/R females had the shortest maximum survival, whereas the R/R males had the longest, a $2 \times 2 \times 2$ factorial analysis of variance was performed on the maximum survival times, with sex, the preweaning diet, and the postweaning diet as factors. The largest of the seven effects in the analysis was the sex by preweaning diet interaction, for which a value of $t = 2.94$, or $p = 0.04$, was calculated. (The standard deviation of the maximum survival of a group of 15 animals was estimated to be 8.2 weeks, on the basis of the fitted Gompertz curves, described below.) Preweaning restriction thus had a significantly different effect on the maximum survival times of the two sexes in cohort I.

Survival distributions have been described indirectly by their age-specific mortality rates, estimates of which can be obtained by fitting the Gompertz function,

$$g(t) = ae^{bt}, \tag{1}$$

where $g(t)$ is the age-specific mortality rate, t is the age at death, and a and b are parameters. Furthermore, an expression describing the distribution for age at death, the experimentally observed variable, can be derived from the Gompertz function, and accordingly, we have estimated the parameters by applying the method of maximum likelihood to age at death

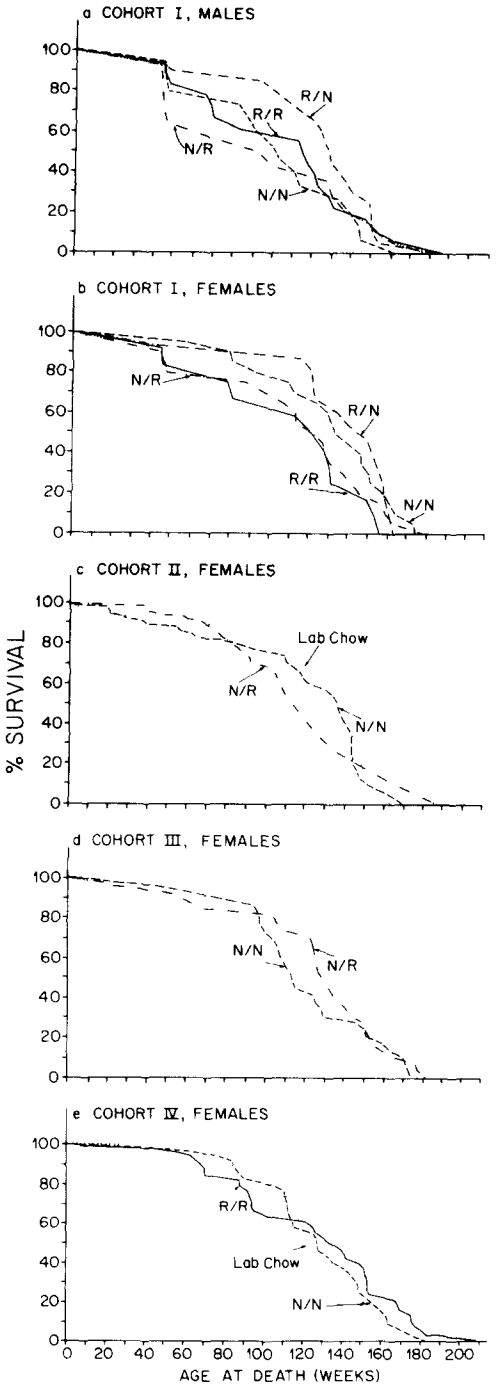


FIG. 1a–e. Survival curves for groups of C57BL/6J mice in four cohorts undernourished pre- and/or postweaning, or fed normally. Product-limit analysis was employed, with neonatal deaths prior to 8 weeks being excluded, and correction factors being applied for any accidental deaths, and for animals culled for experiments

TABLE 3. SUMMARY OF SURVIVAL DATA IN FOUR COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS

Cohort	Diet	Sex	Number of mice		Survival (weeks)		
			Total	Natural deaths (%)*	Mean ± S.E.	50%	Longest-lived survival
I	N/N	M	15	15 (100)	103.2 ± 9.8	103.1	162.6
	R/N	M	20	20 (100)	124.2 ± 7.7	128.9	186.4
	N/R	M	14	14 (100)	96.9 ± 13.1	96.2	178.4
	R/R	M	21	18 (85.7)	106.0 ± 10.1	115.7	187.4
I	N/N	F	20	20 (100)	131.5 ± 7.4	137.3	175.0
	R/N	F	15	15 (100)	137.8 ± 8.1	150.3	163.7
	N/R	F	21	20 (95.2)	115.5 ± 10.9	125.8	181.3
	R/R	F	14	12 (85.7)	111.9 ± 11.5	125.4	157.1
II	Lab Chow	F	48	44 (91.7)	119.8 ± 5.5	128.5	163.9
	N/N	F	100	37 (37.0)	120.2 ± 8.6	84.9†	170.0
	N/R	F	100	42 (42.0)	121.0 ± 6.2	85.6†	187.3
III	N/N	F	75	32 (42.7)	114.7 ± 8.7	127.1	174.6
	N/R	F	75	31 (41.3)	115.3 ± 7.5	113.4	182.0
IV	Lab Chow	F	66	63 (95.5)	127.4 ± 5.2	121.9	156.4
	N/N	F	66	34 (51.5)	127.5 ± 7.1	127.6	182.6
	R/R	F	76	41 (54.0)	122.7 ± 2.4	133.4	204.0

*Excludes animals culled for experiments and any accidental deaths.

†About 50 mice were sacrificed during the interval of 100–115 weeks, skewing the survival distribution to the left.

data. A computer program was prepared to calculate the estimates and their uncertainties (standard errors), and, as an indication of goodness-of-fit, the Cramer-von Mises w^2 statistic. To obviate the problem of censoring and to obtain a good fit, only the latter portions of the distributions were fitted, where $t \geqslant 120$ weeks. This truncation seemed justifiable, particularly since we were interested in examining differences in long-lived survival.

For our purposes, we substituted $a = 1/\alpha$ and $b = 1/\beta$ in equation (1).

Thus,
$$g(t) = 1/\alpha\ e^{t/\beta}. \tag{2}$$

Equation (2) can also be defined as
$$g(t) = f(t)/S(t),$$

where
$$S(t) = \exp\left\{-\frac{\beta\,(e^{t/\beta}-1)}{\alpha}\right\},$$

and
$$f(t) = -\frac{dS(t)}{dt} = \frac{1}{\alpha}\exp\left\{\frac{t}{\beta} - \frac{\beta(e^{t/\beta}-1)}{\alpha}\right\}. \tag{3}$$

The death density function, $f(t)$, describes the distribution of t, the age at death. The conditional distribution of t, given that $t \geqslant t_o > 0$ expressed as

$$f(t/t_o) = \frac{e^{t_o/\beta}}{\alpha}\exp\left\{\frac{(t-t_2)}{\beta} - \frac{\beta\, e^{t_o/\beta}(e^{(t-t_o)/\beta}-1)}{\alpha}\right\},\ t \geqslant t_o > 0. \tag{4}$$

Substituting
$$t' = t - t_0$$

and
$$\alpha' = \alpha \exp(-t_o/\beta)$$

equation (4) becomes
$$f(t') = \frac{1}{\alpha'}\exp\left\{\frac{t'}{\beta} - \frac{\beta(e^{t'/\beta}-1)}{\alpha'}\right\}. \tag{5}$$

TABLE 4. MAXIMUM LIKELIHOOD ESTIMATES OF THE GOMPERTZ PARAMETERS AND GOODNESS-OF-FIT IN FOUR COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS

Cohort	Sex	Diet	N ($t \geq 120$ weeks)	$\hat{\alpha}' \pm$ S.E.	$\hat{\beta}$	Cramer–Von Mises Goodness-of-Fit (w^2)*
I	M	N/N	5	47.8 ± 30.7	25.0	0.0988
	M	R/N	14	52.8 ± 9.2	25.0	0.1165
	M	N/R	6	68.8 ± 24.7	25.0	0.0503
	M	R/R	8	74.5± 18.1	25.0	0.1666
	F	N/N	14	73.9 ± 23.7	25.0	0.0252
	F	R/N	13	60.4 ± 24.5	25.0	0.1553
	F	N/R	11	66.1 ± 17.9	25.0	0.0471
	F	R/R	7	37.7 ± 16.7	25.0	0.0697
II	F	Lab Chow	30	33.2 ± 7.4	25.0	0.1223
	F	N/N	12	53.9 ± 20.6	25.0	0.1620
	F	N/R	3	148.9 ± 91.0	25.0	0.0396
III	F	N/N	21	61.8 ± 12.9	25.0	0.1335
	F	N/R	13	97.3 ± 29.6	25.0	0.0602
IV	F	Lab Chow	37	24.4 ± 4.1†‡	25.0	0.1559
	F	N/N	19	75.6 ± 18.3	25.0	0.0214
	F	R/R	24	122.9 ± 21.1	25.0	0.0671

*$p = 0.05$ when $w^2 = 0.2215$; $p = 0.10$ when $w^2 = 0.1745$; $p = 0.50$ when $w^2 = 0.0738$. A significant p-value would indicate a poor fit.
†$p < 0.0005$ for $\hat{\alpha}'$ Lab Chow vs $\hat{\alpha}'$ N/N' based on the F-test. (See text.)
‡$p < 0.0005$ for $\hat{\alpha}'$ Lab Chow vs $\hat{\alpha}'$ R/R' based on the F-test. (See text.)

In fitting our data to equation (5), we discovered that $\beta = 25$ rendered a good fit to the data for every dietary group, leaving α' as the sole parameter to be estimated. With β fixed at 25, the values for $\hat{\alpha}'$ are given in Table 4.

Graphical forms of the fitted curves are shown in Fig. 2, where

$$\ln S(t') = -1/\alpha'[\beta(e^{t'/\beta} - 1)]. \tag{6}$$

If

$$\tau = \beta(e^{t'/\beta} - 1),$$

then

$$\ln S(\tau) = -1/\alpha'\tau. \tag{7}$$

A straight line is accordingly generated for each group, using equation (7), with a slope of $-1/\alpha'$. The abscissa of the plots is τ, where τ represents a transformed time scale. Each animal thus has its own value for τ, and it can be shown mathematically that $\hat{\alpha}' \equiv \tau$. Unlike the survival times expressed on the real time scale, t, the values expressed on the τ scale have a negative exponential distribution. One useful consequence is that estimates of α' for two populations can be compared using an F-test; i.e. if $\alpha_1' = \alpha_2'$, $\hat{\alpha}_1'/\hat{\alpha}_2'$ is distributed as F, with $2n_1$ and $2n_2$ degrees of freedom. Significant differences in $\hat{\alpha}'$ were noted only in cohort IV, where N/N > Lab Chow ($p < 0.005$) and R/R > Lab Chow ($p < 0.005$) (Table 4). However, except for the females of cohort I, $\hat{\alpha}'$ was numerically larger in the restricted groups than in the normally-fed groups, in every case. A p-value of 0.05 was calculated for this effect, based on a combined test of significance (Fisher, 1970).

Weight curves are shown in Fig. 3. Weights between animals normally-fed and restricted postweaning were well separated throughout life, and were generally significantly different ($p < 0.01$) at any given weighing, as judged by the t-test. Also, a greater separation was evident in the later cohorts, probably reflecting better standardization of the portions as the experiment progressed. In cohort I, the effects of preweaning and/or postweaning

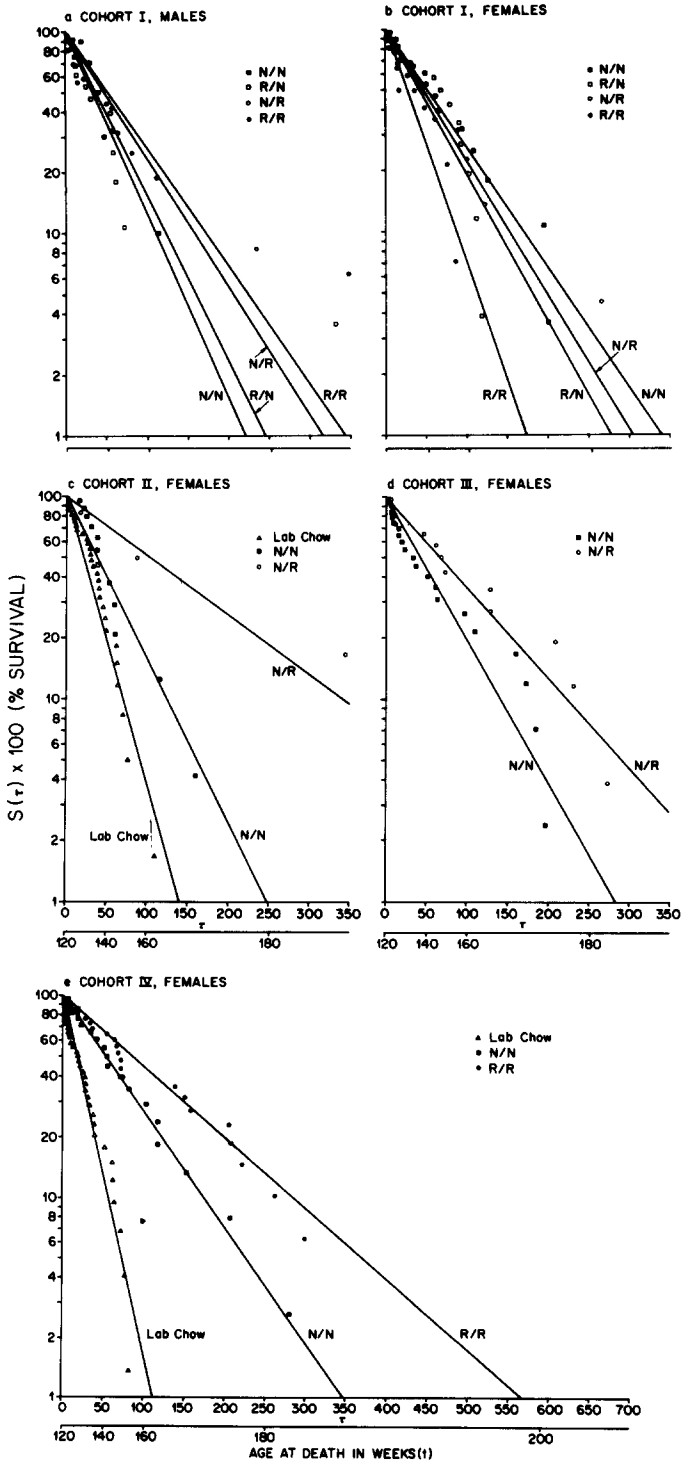


FIG. 2a–e. Plates of the Gompertz function, $\ln S(\tau) = -1/\alpha'\tau$, illustrating the effects of undernutrition on later survival in four cohorts of C57BL/6J mice. $\tau = \beta(e^{t'/\beta} - 1)$, where $t' = t - t_0$ and $t_0 = 120$ weeks. $S(\tau) = (n - i + 1)/(n + 1)$, thus avoiding a zero value when all animals are dead.

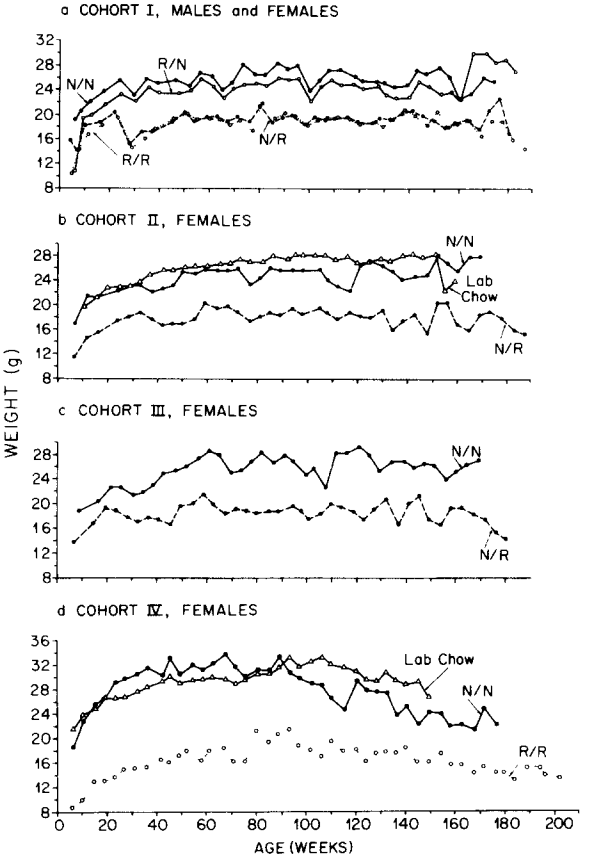


FIG. 3a–e. Comparison of weight throughout life in four cohorts of C57BL/6J mice on various restricted and nonrestricted diets. Standard errors were usually small, with respect to differences among groups, and are not shown for the sake of clarity. (Separate data were not kept for males and females in cohort I.)

restriction could be assessed. The R/N animals apparently never quite "caught up" to the N/N animals, although growth was very rapid in the early postweaning period. Throughout life, the N/N and R/N weights were often significantly different at any given age, even though absolute differences were small. The N/R and R/R groups, on the other hand, showed no weight differences past the early postweaning period, during which time the N/R animals lost weight, thereby approaching the R/R mean weight. In cohorts II and IV, the N/N groups roughly paralleled the Lab Chow controls, except that more fluctuations seemed evident, with some differences being statistically significant. Possibly some inconsistencies in N/N portion sizes were the cause.

Table 5 summarizes the pathology findings for the three cohorts of mice studied for this purpose. In the first cohort, no significant differences were noted between males and females on the same diets. However, both the N/R and R/R groups had a greater percentage of animals with no diseases or tumors at autopsy than either the N/N or R/N groups. This trend was more pronounced and more statistically significant among the females than among the males (Table 6). Similarly, the N/R group in cohort II had significantly greater numbers of animals with no diseases or tumors than the N/N and Lab Chow groups, as did the R/R group in cohort IV, with respect to the Lab Chow group. The N/N and R/R groups in cohort IV were not significantly different in this respect, however, although the trend was in this direction. Fewer than one quarter of the

TABLE 5. SUMMARY OF DISEASE FINDINGS IN THREE COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS*

Cohort	Diet	Sex	Total	Autopsied (%)	No disease(s) or tumor(s) (%)	Disease(s) only no tumors (%)	Tumor(s) only (%)	Tumor(s) and other diseases	Total Tumors (%)
I	N/N	M	15	15 (100)	5 (33.3)	1 (6.67)	9 (60.0)	0 (0.00)	9 (60.0)
	R/N	M	20	18 (90.0)	7 (38.9)	3 (16.7)	3 (16.7)	5 (27.8)	8 (44.4)
	N/R	M	14	13 (92.9)	9 (69.2)	3 (23.1)	1 (7.69)	0 (0.00)	1 (7.69)
	R/R	M	21	18 (85.7)	11 (61.1)	4 (22.2)	1 (5.55)	2 (11.1)	3 (16.7)
I	N/N	F	20	20 (100)	3 (15.0)	1 (5.00)	12 (60.0)	4 (20.0)	16 (80.0)
	R/N	F	15	15 (100)	1 (6.67)	3 (20.0)	3 (20.0)	8 (53.3)	11 (73.3)
	N/R	F	21	19 (90.5)	13 (68.4)	2 (10.5)	4 (21.1)	0 (0.00)	4 (21.1)
	R/R	F	14	12 (85.7)	6 (50.0)	3 (25.0)	3 (25.0)	0 (0.00)	3 (25.0)
II	Lab Chow	F	48	8 (16.7)	4 (50.0)	0 (0.00)	3 (37.5)	1 (12.5)	4 (50.0)
	N/N	F	100	24 (24.0)	17 (70.8)	1 (4.17)	6 (25.0)	0 (0.00)	6 (25.0)
	N/R	F	100	37 (37.0)	37 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
IV	Lab Chow	F	66	63 (95.5)	11 (17.5)	11 (17.5)	31 (49.2)	10 (15.9)	41 (65.1)
	N/N	F	66	33 (50.0)	10 (30.3)	6 (18.2)	12 (36.4)	5 (15.2)	17 (51.5)
	R/R	F	76	40 (52.6)	20 (50.0)	9 (22.5)	3 (7.50)	8 (20.0)	11 (27.5)

*Cohort III was excluded, due to insufficient data. (See Methods and Procedure.)

TABLE 6. STATISTICAL COMPARISON MATRICES FOR THE FREQUENCY OF MICE HAVING NO DISEASE(S) AND/OR TUMOR(S) IN THREE COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS*†

Cohort I, Sex M

Diet	N/N	R/N	N/R	R/R
N/N	5/15			
R/N		7/18		
N/R			9/13	
R/R				11/18

Cohort II, Sex F

Diet	Lab Chow	N/N	N/R
Lab Chow	4/8		0.0004
N/N		17/24	0.0092
N/R			36/37

Cohort I, Sex F

Diet	N/N	R/N	N/R	R/R
N/N	3/20		0.0022	0.0844
R/N		1/15	0.0010	0.0348
N/R			13/19	
R/R				6/12

Cohort IV, Sex F

Diet	Lab Chow	N/N	R/R
Lab Chow	11/63		0.0010
N/N		10/33	0.1434
R/R			20/40

*Cohort III was excluded due to insufficient data. (See Methods and Procedure.)

†p-values given are for the chi square test, with only significant values and trends towards significance being reported. Frequencies for the groups are shown on the diagonals of the matrices.

animals in any group had disease(s) alone, not associated with a tumor(s) (Table 5), and there were not significant differences among the groups in terms of frequency or types of disease. The most common diseases occurring either alone or in conjunction with a tumor(s) were pneumonia, emphysema, and renal disease, excluding pyelonephritis. The only significant association between a particular disease and a particular tumor was in the R/N group of cohort I, where 4 of 7 and 7 of 10 lymphomas in the males and females, respectively, were associated with renal disease, excluding pyelonephritis.

With regard to tumors, in cohort I there were much lower frequencies in the N/R and R/R groups than in the N/N and R/N groups (Table 7). These differences were significant in the females, and the same trend prevailed in the males, where the differences with respect to N/N were significant, and with respect to R/N were nearly so (Table 8). Similarly, the N/R group in cohort II had significantly fewer tumors than both the N/N and Lab Chow groups, as did the R/R group in cohort IV with respect to Lab Chow, and nearly so with respect to N/N. Among the tumor types present, lymphoma predominated, and was the only type differing in frequency among the groups. There were significantly fewer lymphomas, or nearly so, in the N/R and R/R groups than in the N/N and R/N groups for both males and females in cohort I (Table 9). Similarly, the N/R group in cohort II had no lymphomas, which was significantly fewer than in either the N/N or Lab Chow groups. The same trend prevailed in cohort IV, where the R/R had significantly fewer tumors than Lab Chow ($p < 0.0001$), and nearly so with respect to N/N ($p = 0.051$). In this cohort, N/N also had significantly fewer tumors than Lab Chow ($p = 0.0066$). Differences were noted, too, in the age-specific incidence of lymphoma, particularly in cohort IV, where the R/R animals with lymphoma had a significantly greater mean age

TABLE 7. SUMMARY OF TUMOR DATA IN THREE COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS*

Cohort	Diet	Sex	Total(%)	Lymphoma (%)	Hepatoma (%)	Lung (%)	Lymphoma and hepatoma (%)	Lymphoma and lung (%)	Other (%)
I	N/N	M	9 (60.0)	8 (53.3)	0	1 (6.67)	0	0	0
	R/N	M	8 (44.4)	5 (27.8)	0	1 (5.56)	1 (5.56)	1 (5.56)	0
	N/R	M	1 (7.69)	0	1 (7.69)	0	0	0	0
	R/R	M	3 (16.7)	2 (11.1)	1 (5.56)	0	0	0	0
I	N/N	F	16 (80.0)	14 (70.0)	2 (10.0)	0	0	0	0
	R/N	F	11 (73.3)	10 (66.7)	1 (6.67)	0	0	0	0
	N/R	F	4 (21.1)	3 (15.8)	1 (5.26)	0	0	0	0
	R/R	F	3 (25.0)	3 (25.0)	0	0	0	0	0
II	Lab Chow	F	4 (50.0)	4 (50.0)	0	0	0	0	0
	N/N	F	6 (25.0)	6 (25.0)	0	0	0	0	0
	N/R	F	0	0	0	0	0	0	0
IV	Lab Chow	F	41 (65.1)	37 (58.7)	0	1 (1.59)	1 (1.59)	0	1 (1.59) uterine sarcoma 1 (1.59) benign tumor
	N/N	F	11 (33.3)	9 (27.3)	4 (12.1)	4 (12.1)	0	0	0
	R/R	F	11 (27.5)	3 (7.50)	5 (12.5)	2 (5.00)	0	0	1 (2.50) other malignant tumor

*Cohort III was excluded due to insufficient data. (See Methods and Procedure.)

TABLE 8. STATISTICAL COMPARISON MATRICES FOR THE FREQUENCY OF MICE HAVING A TUMOR(S) IN THREE COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS*†

Cohort Sex Diet

I M	N/N	R/N	N/R	R/R
N/N	9/15		0.0129	0.0269
R/N		8/18	0.0682	0.1478
N/R			1/13	
R/R				3/18

Cohort Sex Diet

II F	Lab Chow	N/N	N/R
Lab Chow	4/8		0.0000
N/N		6/24	0.0057
N/R			0/37

I F	N/N	R/N	N/R	R/R
N/N	16/20		0.0008	0.0070
R/N		11/15	0.0069	0.0349
N/R			4/19	
R/R				3/12

IV F	Lab Chow	N/N	R/R
Lab Chow	41/63		0.0004
N/N		17/33	0.0631
R/R			11/40

*Cohort III was excluded due to insufficient data. (See Methods and Procedure.)

†p-values given are for the chi square test, with only significant values and trends toward significance being reported. Frequencies for the groups are shown on the diagonals of the matrices.

at death than the N/N animals with lymphoma ($p = 0.018$), and nearly so with respect to Lab Chow ($p = 0.076$) (Table 9, Fig. 4). Also, in cohort I, the R/N animals with lymphoma tended to have later ages at death than the N/N animals with lymphoma (Fig. 4), although mean age was not significantly different (Table 9).

TABLE 9. STATISTICAL COMPARISON MATRICES FOR FREQUENCY AND AGE-SPECIFIC INCIDENCE OF LYMPHOMA IN THREE COHORTS OF C57BL/6J MICE FOR RESTRICTED AND NONRESTRICTED GROUPS*†

Cohort Sex Diet — Frequency

I M	N/N	R/N	N/R	R/R
N/N	8/15		0.0070	0.0246
R/N		7/18	0.0340	0.1237
N/R			0/13	
R/R				2/18

Mean age at death

	N/N	R/N	N/R	R/R
N/N	116.0 ± 7.9	<0.10		
R/N		138.08‡ ± 5.6		
N/R			-----	
R/R				137.4 ± 24.7

TABLE 9—continued

Cohort I, Sex F

Frequency

Diet	N/N	R/N	N/R	R/R
N/N	14/20		0.0020	0.0354
R/N		10/15	0.0075	0.0775
N/R			3/19	
R/R				3/12

Mean age at death

	N/N	R/N	N/R	R/R
N/N	131.2 ± 8.0	0.171		
R/N		145.2 ± 5.8		0.119
N/R			106.1 ± 32.4	
R/R				109.0 ± 14.6

Cohort II, Sex F

	Lab Chow	N/N	N/R
Lab Chow	4/8		< 0.001
N/N		6/24	0.0057
N/R			0/37

	Lab Chow	N/N	N/R
Lab Chow	86.3 ± 13.1		
N/N		74.9 ± 10.0	
N/R			- - - -

Cohort IV, Sex F

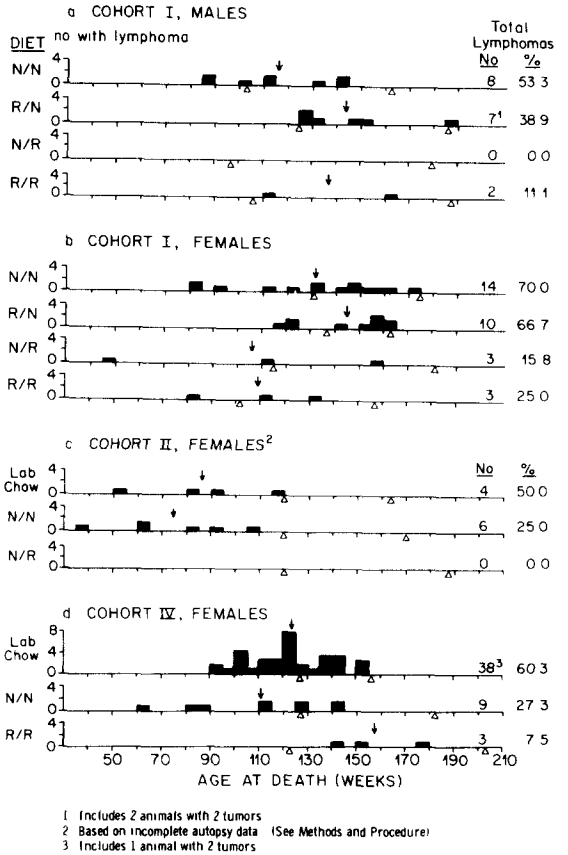
	Lab Chow	N/N	R/R
Lab Chow	38/63	0.0066	< 0.0001
N/N		9/33	0.0510
R/R			3/40

	Lab Chow	N/N	R/R
Lab Chow	123.0 ± 2.7		0.076
N/N		111.0 ± 9.4	0.018
R/N			157.1 ± 10.4

*Cohort III was excluded due to insufficient data. (See Methods and Procedure.)

†*p*-values given are for the chi square test, comparing frequency of lymphoma, and for the *t*-test, comparing age-specific incidence. Only significant values and trends toward significance are reported. Frequencies and mean ages at death ± S.E.M. for each group are shown on the diagonals of the matrices.

‡Excludes two animals with two tumors each. (See Table 7.)



1 Includes 2 animals with 2 tumors
2 Based on incomplete autopsy data (See Methods and Procedure)
3 Includes 1 animal with 2 tumors

FIG. 4a–d. Age-specific distribution of lymphoma within three cohorts of C57BL/6J mice, illustrating the effects of undernutrition on mean age and frequency of lymphoma. The arrows (↓) above the distributions represent the mean age at death of the mice with lymphoma, whereas those arrows (Δ) below the baselines represent the mean and maximum ages at death for the whole group. Cohort III was excluded due to incomplete autopsy data (See Methods and Procedure.)

DISCUSSION

The mouse populations described herein were part of a larger study designed to assess the effects of undernutrition on immune function, as well as on lifespan and disease patterns. Much of the underlying rationale has been considered previously (Walford *et al.*, 1973/4; Walford *et al.*, 1975; Gerbase-DeLima *et al.*, 1975). Thus, whereas the classic experiments in undernutrition have employed the rat (e.g. McCay *et al.*, 1935; Ross, 1961), inbred mouse strains are genetically more homogeneous and are better characterized immunologically, particularly with respect of age (Kay, 1979). Also, long-lived strains such as C57BL/6J are thought to undergo "normal senescence", as opposed to short-lived autoimmune-susceptible strains, e.g. NZB/NZW, which may undergo accelerated aging (Paffenholz, 1978).

Inasmuch as the mouse is more mature at weaning than the rat, at least on a weight basis, preweaning restriction was instituted in some groups by assigning restricted mice to litters of nine and/or by separating them from the mothers every day after day 2 (cohort I) or day 7 (cohort IV). On the other hand, the normally-fed mice were with the mothers continuously, and were assigned to litters of five in cohort IV. Weaning was delayed

slightly in the later cohorts to improve early survival in the groups restricted pre- and/or postweaning (Table 1). Animals were singly caged after weaning, and the level of post-weaning restriction was fixed high enough, hopefully, to extend lifespan. In fact, a single missed feeding often meant death in a restricted mouse, but not in a normally-fed mouse. Significantly, the postweaning restricted diet was designed to prevent malnutrition by providing a normal complement of vitamins and salts. Initially, we had to contend with pelleted diets, with pellets of non-uniform size. Later, we obtained pellets of more uniform size, and still later, we switched to powdered diets, designed to better the uniformity of the portions. Corn oil was substituted for cottonseed oil in cohort IV, when it was learned that gossypol, a potentially toxic pigment in cottonseed, may not be totally removed during processing (Ross, personal communication). Also, in order to provide a better basal diet (Ross, personal communication), the relative proportions of salts and choline were increased in the diets of cohort IV, vs those of the first three cohorts, at the expense of cellulose and cornstarch. (Thus, for the normocaloric diet, salts were increased from 40 to 60 g/kg, whereas choline was increased from 1.0 to 2.5 g/kg, with quantities being doubled in the restricted diets.) These modifications, we believe, may have resulted in the improved survival of our later groups.

The aforesaid dietary changes, e.g. the substitution of corn oil for cottonseed oil and powder for pellets, were undertaken to correct what we perceived as deficiencies in our experimental design. Nonetheless, they introduced new variables. Furthermore, they illustrate a point, which, although largely self-evident, deserves mention: namely, that despite a large measure of correspondence, the cohorts were not entirely comparable. Dietary differences notwithstanding, minor genetic and environmental dissimilarities may have existed, since the cohorts were not wholly contemporaneous. In addition, group sample sizes and the timing and extent of culling varied among cohorts, according to the exigencies of the immune function experiments, reported elsewhere (Walford *et al.*, 1973/4; Walford *et al.*, 1975; Gerbase-DeLima *et al.*, 1975), but were held fairly constant within cohorts (with the exception of the Lab Chow groups, which were not culled).

These discrepancies should not overshadow the basic similarities among cohorts in diet, portions, and feeding schedules. Parallels are very apparent in the results, also, where restriction from the time of weaning or before consistently resulted in fewer lymphomas, the predominant tumor type. In fact, without exception, the N/R and R/R groups had fewer than half the lymphomas of the N/N, R/N, and Lab Chow groups (Table 9). Comparisons of the mean ages at death for animals with lymphoma were more tentative, however, at least in part due to the small numbers of lymphomas in the restricted groups. In cohort I, the age-specific incidence of lymphoma was shifted to the right in the R/N animals with respect to the N/N (Fig. 4), although mean age was not significantly different (Table 9). In cohort IV, the R/R animals with lymphoma did have a significantly greater mean lifespan than the N/N animals. Although overall mean lifespan did not differ among the dietary groups, maximum lifespan was consistently greatest in the most restricted group within each cohort, except in cohort I ($p = 0.04$, according to a signed-rank test). An increase in maximum survival is meaningful, since it is a parameter fairly insensitive to environmental factors such as infectious disease (Walford, 1969), and to the small differences in group size that characterize our data (Sacher, 1959). In cohort I, although the greatest maximum lifespan was in the R/R males, the shortest was in the R/R females (Table 3), resulting in a value of $p = 0.04$ for the sex by preweaning diet interaction in a $2 \times 2 \times 2$ factorial analysis of variance of maximum survival.

The R/R females in cohort I may have been adversely affected by preweaning restriction that was too severe, since the R/R females in cohort IV benefited from preweaning restriction that was somewhat less severe (Tables 1 and 3). Why the males of cohort I showed an extension of maximum survival is unclear, except that if perhaps they were somewhat bigger than the females, they may have been able to obtain more milk, and thus may have been less severely restricted.

In an effort to better describe our survival distributions, we employed the Gompertz function, which states that the age-specific mortality rate increases exponentially with age. We elected to fit our data to $f(t)$, the distribution of t, the age at death. Our parameter β is proportional to the doubling time of the mortality rate (Sacher, 1977). Since β could effectively be fixed, the doubling time of the mortality rate, therefore, did not vary among groups, at least when $t_0 = 120$ weeks. In analyzing the data of Ross (1959) and Berg and Simms (1960) for rats, Sacher (1977) concluded that the doubling time of the mortality rate was increased in the restricted rats. However, if only later survivors are considered ($t \geqslant 100$ weeks), doubling times appear not to differ much.

In any case, Sacher (1977) fits $g(t)$, the age-specific mortality rate, which requires that deaths be grouped into intervals. If sample sizes are large, this method is useful, since it allows one to easily determine whether the data fit the Gompertz function, and if not, alternative models may be apparent. For small sample sizes, however, it is preferable to fit $f(t)$, the distribution of ages at death, since each death is considered separately, i.e. information is not lost by grouping deaths into intervals. Thus, we believe $f(t)$ represents a more precise characterization of the data than $g(t)$. Maximum likelihood, our method of choice for estimating the Gompertz parameters, was shown to be superior to weighted least squares, for this purpose (Garg *et al.*, 1970). Also, we tested goodness-of-fit using the Cramer–von Mises w^2 statistic (Mickey *et al.*, 1963), and thus verified that our data conformed to the model.

While not allowing for comparison of earlier survival, truncation at 120 weeks nevertheless did allow for comparison of the most gerontologically relevant portions of the distributions. This was achieved by converting survival times $\geqslant 120$ weeks on the real time scale to times on a transformed scale, τ, such that $\overline{\tau}_1/\overline{\tau}_2$, or $\hat{\alpha}_1/\hat{\alpha}_2$, had an F-distribution, thus permitting statistical comparison. The only significant comparisons were in cohort IV, where both the N/N and R/R groups had significantly greater mean survival times on the τ scale, $\overline{\tau}$ or $\hat{\alpha}$, than Lab Chow ($p < 0.0005$) (Table 4). However, values for $\hat{\alpha}$ were numerically greater in the restricted groups than in those normally fed, except in the females of cohort I ($p = 0.05$, according to a combined test of significance). Also, since $\hat{\alpha}$ is proportional to the mortality rate on the τ scale, mortality rates were thus not significantly different among groups, at least past 120 weeks of age, but were numerically greater in the normally-fed groups than in those restricted (except in the cohort I females).

All or part of the triad of better survival, less disease, and a shift of diseases to later ages has been reported in a number of studies of dietary restriction in both mice and rats. The interpretation of many of these studies (e.g. Berg and Simms, 1960; Fernandes *et al.*, 1976*a*, *b*; Nolen, 1972; Saxton *et al.*, 1944; Tannenbaum, 1942, 1945*a*, *b*; Visscher *et al.*, 1942) is somewhat problematic, however, due to variations and/or ostensible shortcomings in experimental design, with regard to, for example, the degree, length, and timing (within the lifespan) of the restriction; the dietary composition and the nutritional supplementation of the restricted diets; and the uniformity of the portions, i.e. multiple

animals per cage may unequally share limited quantities of food. Also, most of the mouse studies were undertaken to assess the effects of dietary restriction on disease, rather than on lifespan. Consequently, many of the strains used were short-lived and highly disease-prone; in addition, the experiments were often terminated prior to the deaths of the longest-lived survivors.

Despite these limitations, the results reported were oftentimes dramatic, in terms of better survival, fewer tumors, etc., in the restricted animals. Moreover, the well-controlled studies of Ross and Bras in rats established the authenticity of these phenomena in long-lived rodents. Specifically, they confirmed that undernutrition, begun at weaning and continued throughout life, extends survival, reduces the incidence of tumors and other diseases, and shifts their age-specificity to the right on the time scale (Ross, 1961, 1964, 1972; Ross and Bras, 1965, 1971, 1973). Dietary composition was secondary in importance to caloric intake, within a wide range of protein/carbohydrate ratios. A high ratio, however, was most beneficial when calories were restricted (Ross and Bras, 1973). Short-term restriction, e.g. 7 weeks, was also somewhat effective in lowering tumor frequency and raising age-specific incidence (Ross and Bras, 1971), and in increasing life expectancy (Ross, 1972), provided it was begun early in life, preferably at weaning.

Whereas our dietary compositions were similar to those of Ross and Bras (Ross, 1961; Ross and Bras, 1973), in one experiment, Ross's maximum survival for the restricted rats was 234 weeks, vs 128 weeks for those rats fed Purina Lab Chow *ad lib.* (Ross, 1961). Lifespans > 1800 days (257 weeks) have been reported for restricted rats (Ross, 1976). This compares with our best maximum survival of 204 weeks for the R/R mice (cohort IV), 183 weeks for the N/N mice, and 156 weeks for the Lab Chow group. Clearly, our extension of maximum survival was not as dramatic as Ross's. The reasons for this are uncertain. Nevertheless, data from another study by Ross and co-workers (Ross *et al.*, 1976) may be relevant. In this instance, rats were allowed to "self-select" their own diets, beginning at weaning, with regard to calories and composition, within a wide range of protein/carbohydrate ratios. Mathematical analysis disclosed that rats with the slowest growth rates, especially early in life, had the greatest chance for extended survival. Similarly, Goodrick discovered a negative correlation between growth duration and longevity in C57BL/6J mice fed *ad lib.* diets containing either 26% protein (Goodrick, 1977, 1978), or 4% protein (Goodrick, 1978).

Although our mice received limited food, these conclusions may still apply. For example, our weight data reveal that the R/N mice in cohort I nearly doubled their 7-week-old weight within 3 weeks; also, the R/R mice in cohort I nearly doubled their 7-week-old weight within 18 weeks, whereas the R/R mice in cohort IV, the group with the greatest maximum survival, took about twice as long to do the same (Fig. 3). Thus, if Ross's and Goodrick's conclusions apply, survival may have been extended in the R/R animals of cohort IV because their growth was comparatively slow, i.e. portions were increased more slowly after weaning in cohort IV than in cohort I. Fig. 3 also shows that the normal and restricted groups in cohort IV were more separated by weight throughout life than similar groups in the other cohorts, with the least separation being in cohort I. This was probably due to better portion control overall in the later cohorts, which may have helped improve survival, particularly in the restricted mice of the last cohort. A further note with regard to growth is that the R/N animals in cohort I never quite reached the average weight of the N/N mice, despite their extremely rapid growth following weaning—a phenomenon that has also been reported in rats (Widdowson, 1964). While the R/N diet

correlated with an apparent shift to the right in the age-specific incidence of lymphoma (Fig. 4), and with an apparent survival advantage during much of life (Fig. 1), the rapid weight gain in these animals following weaning may have offset any potential for extended maximum survival.

The foregoing remarks suggest that perhaps greater longevity could be obtained through undernutrition by slowing growth further, and by maintaining a significant weight differential between normal and restricted groups. On the other hand, early mortality would likely increase by slowing growth, unless very fine portion control were exerted. Possibly early mortality could be lessened by a higher protein/carbohydrate ratio in the postweaning restricted diet, which might contribute to better long-lived survival, as well (Ross and Bras, 1973). The rationale is that restricted animals may suffer from a protein deficiency, if they are forced to metabolize protein to meet energy requirements. A higher protein/carbohydrate ratio would thus attempt a re-balancing of the diet with respect to protein, just as we have attempted with respect to vitamins and salts, by approximating a normal complement in the restricted diet.

Other factors contributing to improved survival in the last cohort may have been the substitution of corn oil for cottonseed oil and/or the increase in choline and salts content, compared with the first three cohorts. Certainly, the N/N animals of cohort IV, whose average weight generally exceeded that of its counterparts in the other cohorts (Fig. 3), had the greatest survival, compared with the other N/N females (Fig. 1, Table 3). The Gompertz parameter, $\hat{\alpha}$, was also numerically, but not statistically, greater for the N/N mice in cohort IV, than for those N/N mice in the other cohorts (Table 4). Within cohort IV, also, the N/N mice displayed extended maximum survival vs the Lab Chow group (187 vs 156 weeks) (Fig. 1, Table 3), as well as a significantly larger Gompertz parameter, $\hat{\alpha}$ ($p < 0.0005$), than the Lab Chow group (Table 4). The same was not true in cohort II for the N/N mice compared with the Lab Chow group. Given that weight generally paralleled that of the Lab Chow animals (Fig. 3), the survival advantage of the N/N mice in cohort IV seemingly was due to a more beneficial diet than in cohort II.

Also, Kaunitz and Johnson (1975) have demonstrated that the type of dietary fat can affect tumor patterns in rats fed *ad lib.* Indeed, our N/N mice in cohort IV (fed corn oil) had significantly fewer lymphomas than the N/N mice in cohort I (fed cottonseed oil) ($p < 0.005$). The same trend was observed in the R/R mice, although the numbers were too small for significance. The R/R group in cohort IV, however, had 5 hepatomas, whereas the same group in cohort I had none. In addition, within cohort IV, the N/N group had significantly fewer lymphomas than Lab Chow ($p = 0.0066$) (Table 9).

Since the diet of cohort IV was obtained from a different source than that of the previous cohorts, other unknown elements affecting survival and/or disease patterns may have existed, e.g. the amount of pesticide residue, etc., which would have been difficult to identify, as aptly pointed out by Kaunitz and Johnson (1975). Likewise, it is problematic whether the delay in weaning time may have affected survival and disease patterns in the later cohorts. Strain differences may come into play, as well, inasmuch as we are observing improved extension of survival in (C57BL/10Sn × C3H/HeDiSn) F_1 mice on diets identical to those of cohort IV (Weindruch *et al.*, 1979; Cheney *et al.*, in preparation). Restriction begun at mid-life (15 months of age) in these mice is proving beneficial, too, as others have shown in mice, rats and hamsters (Barrow, 1978).

Still unanswered is the question of how undernutrition extends survival. Our studies in this regard have dealt with immune functional parameters, including skin

allograft rejection rates, and responses to B- and T-cell mitogens *in vitro*, and to sheep red blood cells *in vivo* (Walford *et al.*, 1973/74, 1975; Gerbase-DeLima *et al.*, 1975). In general, immune function appeared to mature more slowly and stay "younger" longer in C57BL/6J N/R and R/R mice than in the N/N mice. In other words, the restricted mice generally had lower immune responses early in life, compared with those normally fed. At mid-life, however, a reversal occurred, whereby the restricted mice had higher immune responses than the N/N mice, a pattern that usually continued into later life (Gerbase-DeLima *et al.*, 1975). (Unfortunately, no R/N mice were available for immune function studies.) The data reported here reveal that survival followed an approximately similar pattern, such that mid-life and sometimes early survival was greater in the N/N mice compared with the N/R or R/R mice, with the situation reversing itself during later life. This was particularly evident in cohort IV (Fig. 1). The only qualifier is that the reversal in survival, i.e. the time when the restricted mice consistently showed better survival than those normally fed, occurred always at a later age than the reversal in immune function (e.g. in cohort IV, $\sim$1 year for the immune function reversal [Gerbase-DeLima *et al.*, 1975] vs $>$ 2 years for the lifespan reversal). These results suggest a possible lag time before the beneficial effects of undernutrition, as manifested by immune function advantages, are realized in terms of improved survival. Arguably then, enhanced immune function is a legitimate antecedent, and thereby a conceivable ancillary factor in, if not the cause of, the extension of long-lived survival in undernourished mice (and in their lessened lymphoma frequency and its shift to later ages).

In another study, Ross (1969) found that undernourished rats displayed a "younger" pattern of hepatic enzyme levels, as compared with controls. Hormonal functions, too, may be altered in undernourished animals, although little work has been done in this area. However, Fabris and Piantanelli (Fabris, 1977; Piantanelli and Fabris, 1978) and others have demonstrated relationships in mice between the endocrine and immune systems. Given that immune status is altered by undernutrition, hormonal status may be altered as well. Denckla (1974) has found that the normal age-related decline in minimal O_2 consumption (MOC) is slowed, at least in young rats, by dietary restriction *only* if the restriction is begun before puberty. The MOC is thought to be related to the thyroidal status of an animal, which may be determined in part by the secretion, beginning at puberty, of an as yet unidentified factor from the pituitary (Denckla, 1974). Removing the pituitary in older rats, with replacement of the known essential hormones at physiological levels, reverses the age-related decline in the MOC, and results in more "youthful" response rates for xenograft rejection and carbon clearance (Bilder and Denckla, 1977). Based on these findings, studies of the MOC in undernourished mice should be rewarding, particularly if correlated with age-matched studies of immune function.

Equally germane, perhaps, is the study of DNA repair. Although no work has been done in undernourished animals, Hart and Setlow (1974) have shown an excellent correlation in seven mammalian species between maximum lifespan potential and DNA repair, as judged by the capacity for unscheduled DNA synthesis. In addition, Paffenholz (1978) has established a similar correlation within three strains of mice: NZB, C3H and CBA, whose mean lifespans are about 300, 600 and 900 days, respectively. Moreover, Schneider and co-workers (Schneider *et al.*, 1979) have reported a decline with age in the number of mutagen-induced sister chromatid exchanges (SCE), a probable type of DNA repair, in both mouse and rat bone marrow cells and in mouse spleen cells *in vivo,* and in human fibroblast cultures as a function of both *in vitro* aging (cell passage number) and

in vivo aging (age of the cell culture donor). Also, chromosomal aberrations increased in the older cell populations, in conjunction with the decreased number of mutagen-induced SCE, suggesting that DNA repair was indeed diminished. (Cell population age did not significantly affect baseline levels of SCE.)

With age, chromatin apparently assumes a more condensed state, which may, in part, reflect facultative heterochromatinization (Tas *et al.*, 1979). DNA-protein adducts may increase, as well (Cutler, 1978). DNA repair and other processes may function as "anti-aging", or "life maintenance processes" (LMP), designed to counterbalance the age-related chromatin alterations. Among mammals, the LMP are thought to be similar, but their differential rates of expression are deemed responsible for the respective species rates of aging and maximum lifespan potentials (Cutler, 1976, 1978). Furthermore, both Cutler (1975) and Sacher (1975) have estimated that only a relatively few gene changes have occurred over evolutionary time to lengthen maximum lifespan potential and slow the rate of aging. According to Cutler (1976, 1978), these changes have probably involved regulatory genes controlling the rates of expression of the LMP.

As suggested by Meredith and Walford (1979), the main histocompatibility complex (MHC) may be one such gene regulatory complex in which changes can alter the rate of aging. Evidence to this effect is derived from a study by Smith and Walford (1978) on three sets of congenic mouse strains, each on a different genetic background, and differing only at the H-2 region, the MHC in mice. A wide range of mean and maximum lifespans within each congenic set implied that the MHC can significantly affect aging. No simple correlation was found between the lifespan and tumor data; however, within each set of strains, there was some indication that immune function, particularly T-cell function, was better preserved into later life in the longer-lived strains, based on their responses to mitogens (Meredith and Walford, 1977).

Significantly, perhaps, the situation in congenic mice displayed certain similarities to that in undernourished mice, as compared with normally-fed mice, i.e. both showed differences in maximum lifespan and in immune function. Of course, the differences between the restricted and normally-fed mice were achieved through dietary manipulation, whereas the differences among the congenic strains resulted from genetic manipulation. Is it possible, however, that genetic *expression* may have been altered in the restricted mice to account for the differences in immune function, etc.? If according to Cutler (1976, 1978), the rates of expression of "primary aging processes" and the timing and degree of expression of "life maintenance processes" govern the rate of aging, perhaps optimum undernutrition alters the expression of these processes, thereby slowing the rate of aging. In this regard, it may be instructive to test whether undernutrition alters the expression of any putative "primary aging processes" (e.g. increased heterochromatinization via the formation of disulphide bonds) and/or any putative "life maintenance processes" (e.g. DNA repair). Recently, Davies (1979) has shown that a diet promoting longevity in insects may delay the condensation of their chromatin. Similar experiments may be decisive in determining whether undernutrition exerts its effects via altered gene expression.

SUMMARY

This study reports survival and disease patterns in a long-lived mouse strain subjected to undernutrition. Four cohorts were studied, each composed of two or more groups of mice, each normally-fed or restricted either pre- and/or postweaning. Restriction prior to weaning was effected by limiting access to the mother. Animals restricted postweaning

received a nutritionally complete diet, including a normal complement of vitamins and salts, but were fed only 4 portions/week vs 7 portions/week for those animals normally fed—hence the term *under*-nutrition to differentiate between this and malnutrition.

Comparisons of disease patterns among groups revealed that the incidence of lymphoma, the most prevalent tumor, was uniformly decreased in the groups restricted postweaning, with or without preweaning restriction. In the last cohort, deaths of animals with lymphoma were shifted to later ages in the restricted groups, compared with the normally-fed controls.

Whereas the lymphoma pattern was considerably modified by undernutrition, the effect on overall survival did not seem as dramatic. Gompertzian parameters for survival past 120 weeks were not statistically different, although with one exception, maximum survival and one of the Gompertzian parameters was consistently greater in groups restricted postweaning, compared with those restricted preweaning only, or not at all. Maximum survival is a parameter not unduly influenced by environmental factors such as infectious disease; consequently, this represents a meaningful effect of undernutrition.

Statistically, more significant differences in tumor patterns than in survival suggests that the former are more sensitive to undernutrition than is the latter—at least in this strain of mouse. Greater lifespan prolongation in the restricted animals may be possible through better "fine tuning" of the diet, including improved portion control, particularly in the early postweaning period, to prevent rapid weight gain, and possibly through changes in dietary composition. Finally, it is suggested that undernutrition may exert its effects through an alteration in gene expression.

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