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Age-Related Neoplasia in a Lifetime Study of *Ad Libitum*-Fed and Food-Restricted B6C3F1 Mice*

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Abstract

Longevity, body weight, and age-specific neoplasia were determined in 1,064 B6C3F1 mice as part of a coordinated study of food restriction (FR). Restricted animals were offered 60% of the diet consumed by the *ad libitum* (AL) group. Longevity data were derived from a set of 56 animals of each sex from each diet group, which were examined whenever dead or moribund. For cross-sectional data, a parallel set of 210 animals were sacrificed in groups of 12–15 at 6-mo intervals. Lifetime body weight was reduced in the FR mice approximately proportional to restriction (i.e., 40%). Food restriction increased the age at 50% survival (median) by 36% in both sexes and increased the maximal lifespan (mean age of oldest 10%) by 21.5% in males and by 32.5% in females. In 56 males of the longevity groups, there were 89 neoplasms in the AL subgroup versus 53 in FR; 56 AL females had 100, versus 58 in 55 FR females. Increase in lifespan of the restricted animals was achieved primarily by decrease in incidence and delay of onset of fatal tumors, of which lymphoma was the most prominent. This report catalogs all of the neoplasms (1,103) observed in longevity and cross-sectional groups, by diet, sex, and age. These data add to the existing knowledge base needed for future studies of dietary restriction and aging as well to evaluate nutrition of animals used in bioassays.

Keywords. Lifespan; tumor incidence; cause of death; lymphoma; pituitary neoplasia

Introduction

The Food and Drug Administration's National Center for Toxicological Research (NCTR), the National Institute of Aging (NIA), and the American Institute of Cancer Research (AICR) have been collaborating on several coordinated studies of dietary restriction using multiple genotypes of rodents (i.e., 4 mouse and 3 rat genotypes on 3 different diets under either restricted or *ad libitum* conditions). The objectives of these studies include the following: (a) establishment and validation of biomarkers of aging; (b) establishment and validation of more reasonable methods to examine animal toxicity arising from chemical exposure; (c) the critical examination of specific generic assumptions and models used in the assessment of risk; and (d) the primary focus of this study, the determination of age-specific pathology and longevity information of *ad libitum*–fed and calorically restricted mice. A general description of these studies has been published (6).

While a plethora of data exists on the pathology of the *ad libitum*–fed B6C3F1 mouse used in the 2-yr chronic bioassay for the National Toxicology Program (e.g., see 2, 4), a paucity of data exists on the pathology of this genotype maintained under calorically restricted conditions. This is despite the large number of reports that do exist on the effect of food restriction on the pathology of a number of other murine genotypes (5).

We report here the neoplasia and longevity data for the B6C3F1 mouse maintained with and without food restriction. In addition we also point out the differential effect caloric restriction can have on different tumor types as well as frequency of occurrence and time to tumor. The non-neoplastic lesions of these mice are the subject of a future report.

Materials and Methods

Experimental Design. One thousand sixty-four B6C3F1 mice were used (Table I). The animals were

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TABLE I.—Number of mice in each diet group.

	Males				Females			
	AL		FR		AL		FR	
	No. allocated	No. examined	No. allocated	No. examined	No. allocated	No. examined	No. allocated	No. examined
LDM	56	56	56	56	56	56	56	55
SS	210	73	210	104	210	70	210	94
SDM		116		102		132		116
Total	266	245	266	262	266	258	266	265

weaned at 3 wk of age and placed in the study at 4 wk, assigned randomly to 1 of 2 dietary groups, *ad libitum* (AL) or food-restricted (FR). Once on study, all mice were housed individually and fed *ad libitum* through 13 wk of age to ensure initial growth and development. After 13 wk, the AL animals were continued on their unlimited feeding schedule, while the diet of the FR animals was reduced stepwise over a 2-wk interval to achieve 60% of the AL consumption (described later).

The FR and AL groups were subdivided into 2 groups: (a) a lifetime group from which longevity data was to be derived and (b) a scheduled sacrifice group to be used in cross-sectional evaluation. Animals in the lifetime group were removed from the study only when dead or moribund and were designated LDM (lifetime; dead and moribund). The scheduled sacrifice group was designated SS (scheduled sacrifice), and these animals were removed from the study at scheduled intervals. Those in the SS groups that were removed between scheduled sacrifices when dead or moribund were designated SDM (scheduled; dead and moribund). In the SS group, animal sacrifice began after 12 mo of study and continued at 6-mo intervals thereafter. The experimental design is depicted in Table I. The total number of animals ultimately available for necropsy and microscopic examination was 1,030 out of a total of 1,064, providing the study with an excellent opportunity for comparisons among groups. Of the 34 mice not examined, 21 were allocated for diagnostic evaluation and the remaining 13 mice were too autolytic or not available for examination.

There were 55–56 mice of each sex in the lifetime group and sufficient animals of each sex in the scheduled sacrifice group to assure 13–19 survivors for each sacrifice period until the final sacrifice. The final sacrifice for all AL mice and FR mice was 36 and 48 mo, respectively (Table IX).

Animals and Diet. B6C3F1 hybrid mice of both sexes, produced by the NCTR specific pathogen free (SPF) breeding colony, were allocated to this study. The mice were assigned to the experiment as weanlings. They were housed individually in polycarbonate cages with filter tops and bedded with hardwood chips. NIH-31 open formula diet (Purina Mills, Inc., Richmond, IN) was fed to both diet groups. The mice on the restricted diet received 60% of the food consumed by their AL cohorts. The restriction began at 14 wk (90% of AL), was stepped down to 75% at 15 wk, and then to 60% at 16 wk. The restricted diet was supplemented with vitamins to the level that was available to the AL mice. The room temperature was maintained at 21 ± 3°C and the relative humidity was 50 ± 10%. The room light cycle was 12 hr on and 12 hr off (6).

Pathology. All mice were necropsied, and approximately 45 tissues or organs as well as all gross lesions were collected for microscopic examination. After fixation in 10% neutral-buffered formalin, all tissues were processed routinely and stained with hematoxylin and eosin for histopathological evaluation.

Data Tabulation. The types and numbers of lesions that occurred at each scheduled sacrifice period (age) can be compared directly between the AL and FR groups. To make comparisons of age-related disease processes in dead and moribund mice from the longevity group to those that died spontaneously between sacrifices of the SS group, the data from all animals removed as dead or moribund were tabulated for 6-mo intervals, each interval centering on a sacrifice date. In this way, for example, SDM and LDM animals that were removed between 639 and 821 days can be compared to each other as well as with the SS animals sacrificed at 24 mo (720 days). Therefore, all references to a particular time point (e.g., 24 mo) in the LDM and SDM animals refer to that time point ± 3 mo (Table IX). In this report, each month contains 30.4 days.

The age at 50% survival, the maximal lifespan, and the age at death of the oldest survivor are summarized for each group in Table II. Table III lists by sex, diet group, and age the average number of mouse-days survived by tumor-bearing mice per tumor produced. To compare occurrence of neoplasms among the groups, data in Tables IV–VII were divided into 4 intervals, for animals examined at 0–24, 30, 36, and 42–48 mo. (There were relatively few dead or moribund animals at the 12- and

TABLE II.—Age at 50% survival, maximal lifespan,[a] and oldest single survivor in the B6C3F1 longevity group measured in days (months in parentheses).

	Males		Females	
	AL	FR	AL	FR
50% Survival	979 (32.2)	1,331 (43.8)	922 (30.3)	1,255 (41.3)
Maximal lifespan	1,270 (41.8)	1,543 (50.8)	1,106 (36.4)	1,462 (48.1)
Oldest survivor	1,293 (42.5)	1,568 (51.6)	1,128 (37.1)	1,489 (48.0)

[a] Maximal lifespan = average age, in days, of oldest 10% in each group.

18-mo periods; at 48 mo, there were no surviving AL animals). The percentage of animals with neoplasms (benign or malignant) in each group is summarized in Table IV. The total number of neoplasms per group is depicted in Table V (some animals had multiple tumors). The average number of tumors in tumor-bearing mice is presented by group in Table VI. The respective incidence of the 11 most prevalent neoplasms is summarized by diet group, sex, and age in Table VII. In Table VIII, the overall incidence of specific neoplasms is listed by sex and diet group, without regard to age. Table IX (males and females) lists the number of each neoplasm diagnosed, within diet group and time period.

The data in Table III reveal that on an overall lifetime basis the time required to develop neoplasms in FR animals is approximately 2-fold longer than it is for AL animals. This quantifies the general observation of fewer tumors and later onset of tumors in the FR animals, with longer lifespan an implicit corollary.

RESULTS AND DISCUSSION

Body Weights

In Fig. 1 the weekly mean body weight for each treatment group, derived from the LDM animals as a function of time, is plotted. The FR animals maintained a relatively constant body weight over the course of the study. After about 6.25 mo, the weight of the females held steady at between 22 and 24 g for the rest of the study. The average male body weight was 26 g starting at 10 mo and remained so until 30 mo and then declined gradually to 23.7 g by the end of the study (56 mo). The AL males gained weight rapidly, from 20 to 40 g in the first year, to 43.9 g by the end of the second year, and then declined steadily over the next 20 mo to end with a mean body weight of 31.8 g. The AL females gained less rapidly, peaked at 38.5 g at 2 yr, then declined to 30.1 g by 43.5 mo.

TABLE III.—Mouse-days at risk per neoplasm[a] (tumor-bearing, longevity mice).

	Males		Females	
Months (days)	AL (89/56[b])	FR (53/56)	AL (100/56)	FR (58/55)
0–24 (1–821)	573	1,505	502	627
30 (822–1,003)	750	1,331	557	893
36 (1,004–1,186)	493	989	460	969
42 (1,187–1,368)	628	1,064	612	1,116
48 (1,369–1,568)	—	1,658	—	1,646
Overall	603	1,328	507	1,154

[a] The listed number is the aggregate number of days on study per tumor produced, for animals removed as dead or moribund within the respective time intervals.
[b] Total number of neoplasms/number of animals examined.

Longevity (LDM Animals)

The survival curve for each LDM group is plotted in Fig. 2. These curves are based on a total of 223 animals, all of which died as a result of natural causes; they include 55 female and 56 male FR animals and 56 AL animals of each sex.

Females. Since neoplasia accounted for most deaths (84% of AL animals and 62% of FR animals), it is not possible to separate, in the discussion of our results, longevity from neoplasia. Of all neoplasms, lymphoma was the most common fatal tumor appearing in both dietary groups. It was frequently fatal and was the primary determinant of both survival rate and longevity, in both groups. Of the 38 AL longevity mice that had been removed as dead or moribund through the 30-mo period, lymphoma was the primary cause of death in 25 (65.8%); it also accounted for 3 of 6 deaths (50%) in FR longevity animals at this age. By the end of the study, lymphoma alone or with other neoplasms accounted for 34 of 56 deaths in AL animals (60.7%) and 16 of 55 in FR animals (29.1%). Of all animals with neoplasms in the AL group, lymphoma and histiocytic sarcoma represented 71%, whereas in the FR animals these neoplasms were found in 49% of all animals with neoplasia.

After lymphoma, the next 2 most common neoplasms were pituitary adenomas (30.4%) and thyroid follicular adenomas (14.3%) in AL females (Table VII). In contrast, these 2 neoplasms were among the most infrequently diagnosed in the FR mice, at

TABLE IV.—Number of neoplasm-bearing mice/number of mice examined (%).

Months (days)	Males						Females					
	AL			FR			AL			FR		
	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
0–24 (1–821)	14/43 (32.6)	24/28 (85.7)	9/11 (81.8)	2/42 (4.8)	1/3 (33.3)	2/4 (50.0)	9/41 (22.0)	34/40 (85.0)	11/16 (68.7)	5/42 (11.9)	3/7 (42.9)	3/3 (100.0)
30 (822–1,003)	11/15 (73.3)	46/47 (97.9)	20/21 (95.2)	3/15 (20.0)	8/9 (88.9)	1/3 (33.3)	11/15 (73.3)	72/73 (98.6)	22/22 (100.0)	2/15 (13.3)	16/20 (80.0)	3/3 (100.0)
36 (1,004–1,186)	12/15 (80.0)	32/34 (94.1)	16/16 (100.0)	3/15 (20.0)	12/20 (60.0)	11/13 (84.6)	13/14 (92.9)	19/19 (100.0)	15/17 (88.2)	7/15 (46.7)	32/34 (94.1)	10/15 (66.7)
42–48 (1,187–1,568)[a]	—	7/7 (100.0)	7/8 (87.5)	13/32 (40.6)	44/70 (62.9)	24/36 (66.7)	—	—	1/1 (100.0)	9/22 (40.9)	45/55 (81.8)	23/34 (67.6)
Total	37/73 (50.7)	109/116 (94.0)	52/56 (92.9)	21/104 (20.2)	65/102 (63.7)	38/56 (67.9)	33/70 (47.1)	125/132 (94.7)	49/56 (87.5)	23/94 (24.5)	96/116 (82.8)	39/55 (70.9)

[a] Oldest survivor, a FR male, was removed on day 1568 (48 + 3 mo).

TABLE V.—Number of neoplasms/number of mice examined.

Months (days)	Males						Females					
	AL			FR			AL			FR		
	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
0–24 (1–821)	16/43 (0.37)[a]	31/28 (1.1)	12/11 (1.1)	2/42 (0.05)	1/3 (0.3)	2/4 (0.5)	9/41 (0.2)	46/40 (1.1)	23/11 (1.4)	5/42 (0.1)	4/7 (0.6)	3/3 (1.0)
30 (822–1,003)	17/15 (1.1)	71/47 (1.5)	26/21 (1.2)	3/15 (0.2)	11/9 (1.2)	2/3 (0.7)	24/15 (1.6)	132/73 (1.8)	36/22 (1.6)	2/15 (0.1)	18/20 (0.9)	3/3 (1.0)
36 (1,004–1,186)	21/15 (1.4)	62/34 (1.8)	35/16 (2.2)	4/15 (0.3)	14/20 (0.7)	14/13 (1.1)	40/14 (2.9)	50/19 (2.6)	39/17 (2.3)	9/15 (0.6)	40/34 (1.2)	17/15 (1.1)
42–48 (1,187–1,568)[b]	—	18/7 (2.6)	16/8 (2.0)	16/32 (0.5)	63/70 (0.9)	35/36 (1.0)	—	—	2/1 (2.0)	13/22 (0.6)	61/55 (1.1)	35/34 (1.0)
Total	54/73 (0.7)	182/116 (1.6)	89/56 (1.6)	25/104 (0.2)	89/102 (0.9)	53/56 (0.9)	73/70 (1.0)	228/132 (1.7)	100/56 (1.8)	29/94 (0.3)	123/116 (1.1)	58/55 (1.0)

[a] Number of neoplasms/animal, including tumor-free animals.
[b] Oldest survivor, a FR male, was removed on day 1568 (48 + 3 mo).

TABLE VI.—Number of neoplasms/number of tumor-bearing mice.

Months (days)	Males						Females					
	AL			FR			AL			FR		
	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
0–24	16/14	31/24	12/9	2/2	1/1	2/2	9/9	46/34	23/11	5/42	4/3	3/3
(1–821)	(1.1)[a]	(1.3)	(1.3)	(1.0)	(1.0)	(1.0)	(1.0)	(1.3)	(2.1)	(1.0)	(1.3)	(1.0)
30	17/11	71/46	26/20	3/3	11/8	2/1	24/11	132/72	36/22	2/2	18/16	3/3
(822–1,003)	(1.5)	(1.5)	(1.3)	(1.0)	(1.4)	(2.0)	(2.2)	(1.8)	(1.6)	(1.0)	(1.1)	(1.0)
36	21/12	62/32	36/16	4/3	14/12	14/11	40/13	50/19	39/15	9/7	40/32	17/10
(1,004–1,186)	(1.7)	(1.9)	(2.2)	(1.3)	(1.2)	(1.3)	(3.1)	(2.6)	(2.6)	(1.3)	(1.2)	(1.7)
42–48	—	18/7	16/7	16/13	63/44	35/24	—	—	2/1	13/9	61/45	35/23
(1,187–1,568)[b]		(2.6)	(2.2)	(1.2)	(1.4)	(1.5)			(2.0)	(1.4)	(1.4)	(1.5)
Total	54/37	182/109	89/52	25/21	89/65	53/38	73/33	228/125	100/49	29/23	123/96	58/39
	(1.5)	(1.7)	(1.7)	(1.2)	(1.4)	(1.4)	(2.2)	(1.8)	(2.0)	(1.3)	(1.3)	(1.5)

[a] Average number of tumors/tumor-bearing mouse.
[b] Oldest survivor, a FR male, was removed on day 1568 (48 + 3 mo).

TABLE VII.—Prevalence of major neoplasms.[a]

Neoplasm	Months (days)	Males						Females					
		AL			FR			AL			FR		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
Lung	0–24	7/43	8/28	2/11	1/42	0/3	1/4	1/41	2/40	2/16	1/42	1/7	0/3
	(1–821)	(16.3)	(28.6)	(18.2)	(2.4)	—	(25.0)	(2.4)	(5.0)	(12.5)	(2.4)	(14.3)	—
	30	6/15	9/47	4/21	0/15	1/9	1/3	1/15	10/73	1/22	0/15	2/20	0/3
	(822–1,003)	(40.0)	(19.1)	(19.0)	—	(11.1)	(33.3)	(6.7)	(13.7)	(4.5)	—	(10.0)	—
	36	3/15	20/34	7/16	2/15	4/20	2/13	1/14	1/19	2/17	0/15	5/34	2/15
	(1,004–1,186)	(20.0)	(58.8)	(43.7)	(13.3)	(20.0)	(15.4)	(7.1)	(5.3)	(11.8)	—	(14.7)	(13.3)
	42–48	—	5/7	4/8	4/32	17/70	6/36	—	—	0/1	4/22	8/55	5/34
	(1,187–1,568)[b]		(71.4)	(50.0)	(12.5)	(24.3)	(16.7)			—	(18.2)	(14.5)	(14.7)
Total		16/73	42/116	17/56	7/104	22/102	10/56	3/70	13/132	5/56	5/94	16/116	7/55
		(21.9)	(36.2)	(30.4)	(6.7)	(21.6)	(17.9)	(4.3)	(9.8)	(8.9)	(5.3)	(13.8)	(12.7)
Lymphoma	0–24	0/43	6/28	2/11	1/42	0/3	0/4	1/41	16/40	8/16	1/42	0/7	2/3
	(1–821)	—	(21.4)	(18.2)	(2.4)	—	—	(2.4)	(40.0)	(50.0)	(2.4)	—	(66.7)
	30	3/15	17/47	9/21	0/15	3/9	0/3	4/15	43/73	17/22	0/15	8/20	1/3
	(822–1,003)	(20.0)	(36.2)	(42.9)	—	(33.3)	—	(26.7)	(58.9)	(77.3)	—	(40.0)	(33.3)
	36	6/15	12/34	8/16	0/15	5/20	5/13	9/14	16/19	9/17	1/15	17/34	2/15
	(1,004–1,186)	(40.0)	(35.3)	(50.0)	—	(25.0)	(38.5)	(64.3)	(84.2)	(52.9)	(6.7)	(50.0)	(13.3)
	42–48	—	1/7	4/8	3/32	10/70	1/36	—	—	0/1	2/22	17/55	11/34
	(1,187–1,568)[b]		(14.3)	(50.0)	(9.4)	(14.3)	(2.8)			—	(9.1)	(30.9)	(32.3)
Total		9/73	36/116	23/56	4/104	18/102	6/56	14/70	75/132	34/56	4/94	42/116	16/55
		(12.3)	(31.0)	(41.1)	(3.8)	(17.6)	(10.7)	(20.0)	(56.8)	(60.7)	(4.3)	(36.2)	(29.1)

TABLE VII.—Continued.

Neoplasm	Months (days)	Males						Females					
		AL			FR			AL			FR		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
Histiocytic sarcoma	0–24	0/43	1/28	0/11	0/42	0/3	0/4	0/41	3/40	0/16	0/42	0/7	0/3
	(1–821)	—	(3.6)	—	—	—	—	—	(7.5)	—	—	—	—
	30	0/15	7/47	2/21	0/15	4/9	0/3	0/15	7/73	0/22	0/15	3/20	0/3
	(822–1,003)	—	(14.9)	(9.5)	—	(44.4)	—	—	(9.6)	—	—	(15.0)	—
	36	0/15	2/34	3/16	0/15	2/20	3/13	1/14	3/19	1/17	0/15	2/34	1/15
	(1,004–1,186)	—	(5.9)	(18.8)	—	(10.0)	(23.1)	(7.1)	(15.8)	(5.9)	—	(5.9)	(6.7)
	42–48	—	2/7	0/8	3/32	1/70	4/36	—	—	0/1	0/22	2/55	2/34
	(1,187–1,568)[b]		(28.6)	—	(9.4)	(1.4)	(11.1)			—	—	(3.6)	(5.9)
Total		0/73	12/116	5/56	3/104	7/102	7/56	1/70	13/132	1/56	0/94	7/116	3/55
		—	(10.3)	(8.9)	(2.9)	(6.9)	(12.5)	(1.4)	(9.8)	(1.8)	—	(6.0)	(5.4)
Liver	0–24	7/43	8/28	5/11	0/42	0/3	0/4	2/41	6/40	1/16	0/42	0/7	0/3
	(1–821)	(16.3)	(28.6)	(45.4)	—	—	—	(4.9)	(15.0)	(6.2)	—	—	—
	30	5/15	21/47	6/21	3/15	1/9	0/3	2/15	8/73	0/22	0/15	0/20	0/3
	(822–1,003)	(33.3)	(44.7)	(28.6)	(20.0)	(11.1)	—	(13.3)	(11.0)	—	—	—	—
	36	8/15	14/34	7/16	0/15	0/20	0/13	6/14	1/19	5/17	5/15	1/34	0/15
	(1,004–1,186)	(53.3)	(41.2)	(43.7)	—	—	—	(42.9)	(5.3)	(29.4)	(33.3)	(2.9)	—
	42–48	—	3/7	4/8	0/32	8/70	5/36	—	—	0/1	2/22	4/55	6/34
	(1,187–1,568)[b]		(42.9)	(50.0)	—	(11.4)	(13.9)			—	(9.1)	(7.3)	(17.6)
Total		20/73	46/116	22/56	3/104	9/102	5/56	10/70	15/132	6/56	7/94	5/116	6/55
		(27.4)	(39.7)	(39.3)	(2.9)	(8.8)	(8.9)	(14.3)	(11.4)	(10.7)	(7.4)	(4.3)	(10.9)
Vascular	0–24	1/43	4/28	0/11	0/42	0/3	1/4	0/41	1/40	2/16	0/42	0/7	0/3
	(1–821)	(2.3)	(14.3)	—	—	—	(25.0)	—	(2.5)	(12.5)	—	—	—
	30	2/15	10/47	1/21	0/15	0/9	0/3	1/15	3/73	0/22	0/15	2/20	0/3
	(822–1,003)	(13.3)	(21.3)	(4.8)	—	—	—	(6.7)	(4.1)	—	—	(10.0)	—
	36	1/15	6/34	5/16	0/15	1/20	0/13	0/14	0/19	2/17	0/15	5/34	3/15
	(1,004–1,186)	(6.7)	(17.6)	(31.2)	—	(5.0)	—	—	—	(11.8)	—	(14.7)	(20.0)
	42–48	—	2/7	1/8	1/32	2/70	2/36	—	—	0/1	0/22	7/55	2/34
	(1,187–1,568)[b]		(28.6)	(12.5)	(3.1)	(2.9)	(5.6)			—	—	(12.7)	(5.9)
Total		4/73	22/116	7/53	1/104	3/102	3/56	1/70	4/132	4/56	0/94	14/116	5/55
		(5.5)	(19.0)	(12.5)	(0.96)	(2.9)	(5.4)	(1.4)	(3.0)	(7.1)	—	(12.1)	(9.1)
Musculo-skeletal	0–24	0/43	1/28	0/11	0/42	0/3	0/4	0/41	3/40	1/16	0/42	1/7	0/3
	(1–821)	—	(3.6)	—	—	—	—	—	(7.5)	(6.2)	—	(14.3)	—
	30	0/15	0/47	0/21	0/15	0/9	0/3	0/15	2/73	0/22	0/15	1/20	0/3
	(822–1,003)	—	—	—	—	—	—	—	(2.7)	—	—	(5.0)	—
	36	0/15	0/34	0/16	0/15	0/20	0/13	0/14	0/19	1/17	0/15	2/34	0/15
	(1,004–1,186)	—	—	—	—	—	—	—	—	(5.9)	—	(5.9)	—
	42–48	—	0/7	0/8	1/32	0/70	0/36	—	—	0/1	0/22	2/55	1/34
	(1,187–1,568)[b]		—	—	(3.1)	—	—			—	—	(3.6)	(2.9)
Total		0/73	1/116	0/56	1/104	0/102	0/56	0/70	5/132	2/56	0/94	6/116	1/55
		—	(0.9)	—	(1.0)	—	—	—	(3.8)	(3.6)	—	(5.2)	(1.8)
Skin and sub-cutaneous tissue	0–24	0/43	0/18	1/11	0/42	0/3	0/4	1/41	3/40	0/16	0/42	1/7	0/3
	(1–821)	—	—	(9.1)	—	—	—	(2.4)	(7.5)	—	—	(14.3)	—
	30	0/15	1/47	0/21	0/15	0/9	0/3	0/15	6/73	4/22	0/15	1/20	1/3
	(822–1,003)	—	(2.1)	—	—	—	—	—	(8.2)	(18.2)	—	(5.0)	(33.3)

TABLE VII.—Continued.

Neoplasm	Months (days)	Males						Females					
		AL			FR			AL			FR		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
	36	0/15	0/34	0/16	0/15	0/20	0/13	0/14	1/19	0/17	0/15	3/34	3/15
	(1,004–1,186)	—	—	—	—	—	—	—	(5.3)	—	—	(8.8)	(20.0)
	42–48	—	0/7	0/8	0/32	0/70	0/36	—	—	0/1	0/22	3/55	0/34
	(1,187–1,568)[b]		—	—	—	—	—			—	—	(5.4)	—
Total		0/73	1/116	1/56	0/104	0/102	0/56	1/70	10/132	4/56	0/94	8/116	4/55
		—	(0.9)	(1.8)	—	—	—	(1.4)	(7.6)	(7.1)	—	(6.9)	(7.3)
Kidney	0–24	0/43	0/28	0/11	0/42	0/3	0/4	0/41	0/40	0/16	0/42	0/7	0/3
	(1–821)	—	—	—	—	—	—	—	—	—	—	—	—
	30	0/15	0/47	0/21	0/15	0/9	0/3	0/15	0/73	0/22	0/15	0/20	0/3
	(822–1,003)	—	—	—	—	—	—	—	—	—	—	—	—
	36	0/15	0/34	0/16	1/15	0/20	0/13	0/14	0/19	0/17	0/15	0/34	0/15
	(1,004–1,186)	—	—	—	(6.7)	—	—	—	—	—	—	—	—
	42–48	—	1/7	0/8	2/32	10/70	9/36	—	—	0/1	0/22	3/55	1/34
	(1,187–1,568)[b]		(14.3)	—	(6.3)	(14.3)	(25.0)			—	—	(5.4)	(2.9)
Total		0/73	1/116	0/53	3/104	10/102	9/56	0/70	0/132	0/56	0/94	3/116	1/55
		—	(0.9)	—	(2.9)	(9.8)	(16.1)	—	—	—	—	(2.6)	(1.8)
Pituitary gland	0–24	0/43	0/28	0/11	0/42	0/3	0/4	0/41	1/40	2/16	0/42	0/7	0/3
	(1–821)	—	—	—	—	—	—	—	(2.5)	(12.5)	—	—	—
	30	0/15	0/47	0/21	0/15	0/9	0/3	4/15	12/73	1/22	1/15	0/20	0/3
	(822–1,003)	—	—	—	—	—	—	(26.7)	(16.4)	(4.5)	(6.7)	—	—
	36	0/15	0/34	0/16	0/15	0/20	0/13	3/14	7/19	8/17	0/15	1/34	0/15
	(1,004–1,186)	—	—	—	—	—	—	(21.4)	(36.8)	(47.1)	—	(2.9)	—
	42–48	—	0/7	0/8	0/32	0/70	0/36	—	—	1/1	0/22	2/55	0/34
	(1,187–1,568)[b]		—	—	—	—	—			(100.0)	—	(3.6)	—
Total		0/73	0/116	0/56	0/104	0/102	0/56	9/70	28/132	17/56	0/94	3/116	0/55
		—	—	—	—	—	—	(12.9)	(21.2)	(30.4)	—	(2.6)	—
Thyroid follicular cell	0–24	0/43	0/28	0/11	0/42	0/3	0/4	0/41	1/40	2/16	0/42	0/7	0/3
	(1–821)	—	—	—	—	—	—	—	(2.5)	(12.5)	—	—	—
	30	0/15	0/47	0/21	0/15	0/9	0/3	4/15	12/73	1/22	1/15	0/20	0/3
	(822–1,003)	—	—	—	—	—	—	(26.7)	(16.4)	(4.5)	(6.7)	—	—
	36	0/15	0/34	0/16	0/15	0/20	0/13	1/14	5/19	5/17	0/15	1/34	0/15
	(1,004–1,186)	—	—	—	—	—	—	(7.1)	(26.3)	(29.4)	—	(2.9)	—
	42–48	—	0/7	0/8	0/32	0/70	0/36	—	—	0/1	0/22	2/55	1/34
	(1,187–1,568)[b]		—	—	—	—	—			—	—	(3.6)	(2.9)
Total		0/73	0/116	0/56	0/104	0/102	0/56	5/70	18/132	8/56	1/94	3/116	1/55
		—	—	—	—	—	—	(7.1)	(13.6)	(14.3)	(1.1)	(2.6)	(1.8)
Harderian gland	0–24	0/43	1/28	1/11	0/42	0/3	0/4	0/41	1/40	0/16	2/42	0/7	0/3
	(1–821)	—	(3.6)	(9.1)	—	—	—	—	(2.5)	—	(4.8)	—	—
	30	0/15	3/47	2/21	0/15	1/9	0/3	1/15	6/73	2/22	1/15	0/20	0/3
	(822–1,003)	—	(6.4)	(9.5)	—	(11.1)	—	(6.7)	(8.2)	(9.1)	(6.7)	—	—
	36	2/15	6/34	2/16	0/15	2/20	3/13	5/14	4/19	1/17	1/15	1/34	3/15
	(1,004–1,186)	(13.3)	(17.6)	(12.5)	—	(10.0)	(23.1)	(35.7)	(21.0)	(5.9)	(6.7)	(2.9)	(20.0)
	42–48	—	2/7	0/8	2/32	6/70	3/36	—	—	0/1	0/22	4/55	1/34

TABLE VII.—Continued.

Neoplasm	Months (days)	Males						Females					
		AL			FR			AL			FR		
		SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM	SS	SDM	LDM
	(1,187–1,568)[b]		(28.6)	—	(6.3)	(8.6)	(8.3)			—	—	(7.3)	(2.9)
Total		2/73 (2.7)	12/116 (10.3)	5/56 (8.9)	2/104 (1.9)	9/102 (8.8)	6/56 (10.7)	6/70 (8.6)	11/132 (8.3)	3/56 (5.4)	4/94 (4.3)	5/116 (4.3)	4/55 (7.3)

[a] Number of neoplasms/number of animals examined (%).
[b] Oldest survivor, a FR male, was removed on day 1568 (48 + 3 mo).

0 and 1.8%, respectively. One potential explanation for the decrease in pituitary adenomas may relate to the observation that caloric restriction appears to significantly influence the regulatory mechanisms of the pituitary via depressed production of various hypothalamic neurotransmitters (3). The incidences of the lung, liver, vascular, skin/subcutaneous, and Harderian neoplasms in the AL mice were closely mimicked by the overall incidence of these neoplasms in the FR mice. It appears that the similarity of incidence of these neoplasms between the diet groups can be attributed largely to the extended life of the FR mice, because most of these neoplasms occurred later in them. For other than the strongly hormonally dependent tumors where under FR conditions there was elimination of such tumors, less hormonally dependent tumors were delayed, thus changing the time-to-tumor. In general, neoplasms of the endocrine system were strongly decreased in incidence, and nonendocrine tumors were delayed in onset (time-to-tumor) in the FR mice.

Despite their extended lifespan, the FR mice had fewer total neoplasms; both the multiplicity of tumors per animal and the proportion of tumor-bearing animals were reduced. Overall, the FR animals had 58 separate neoplasms, occurring in 39 of 55 mice in this group (71%), with an average of 1.5 tumors per tumor-bearing animal. In the AL group 49 of 56 mice (88%) had 100 neoplasms, an average of 2.0 each. The incidence of histiocytic sarcoma in the female FR mice was 5.4 versus 1.8% in the AL mice.

Males. Longevity of the males exceeded that for females in both diet groups (Fig. 2). At the end of the 30-mo period (33 mo), 24 of 56 AL males (42.8%) and 49 of the 56 FR males (87.5%) remained. In AL animals, lymphomas and neoplasms of the liver, lung, and vasculature were frequently associated with morbidity and mortality. Alone or in concert with other neoplasms, these tumors were the primary determinants of the shorter lifespan of the AL longevity males. Neoplasia caused the death of 87% of the AL-fed mice and 38% of those that were FR. In AL mice these neoplasms were responsible for the death of 24 of the 32 animals removed during the 12–30-mo periods, but for only 1 of the 7 FR mice. Other indicators of the magnitude of the neoplastic response in the male AL versus FR mice were the respective percentage of mice with tumors (93 vs 68%), total number of tumors (89 vs 53) and the average number of tumors in mice with tumors (1.7 vs 1.4).

Sixty-four percent of the FR mice survived beyond the 36-mo period, versus 14% of the AL mice. This extended longevity to the 42- or 48-mo period was not accompanied by any significant changes from

TABLE VIII.—Overall incidence of specific neoplasms in sacrificed and dead or moribund mice.

	Males				Females			
	AL		FR		AL		FR	
Neoplasm	No.	%	No.	%	No.	%	No.	%
Hepatocellular	88	35.9	17	6.5	31	12.0	18	6.8
Alveolar-bronchiolar (lung)	75	30.6	39	14.9	21	8.1	28	10.6
Lymphoma	68	27.8	28	10.7	123	47.7	62	23.4
Vascular	33	13.5	7	2.7	9	3.5	19	7.2
Histiocytic sarcoma	17	6.9	17	6.5	15	5.8	10	3.8
Harderian gland	19	7.8	17	6.5	20	7.8	13	4.9
Pituitary	0	—	0	—	54	20.9	3	1.1
Thyroid follicular cell	0	—	0	—	31	12.0	5	1.9
Musculoskeletal	1	0.4	1	0.4	7	2.7	7	2.6
Skin and subcutaneous	2	0.8	0	—	15	5.8	12	4.5
Kidney	1	0.4	22	8.4	0	—	4	1.5
Mammary gland (malignant)	0	—	0	—	26	10.0	1	0.9
All others	21	8.6	19	7.3	49	19.0	28	10.6
Total neoplasms	325		167		401		210	

the 36-mo mice with respect to the percentage or number of tumor-bearing mice (Table IV), number of neoplasms (Table V), or number of neoplasms per tumor-bearing mouse (Table VI). However, the types of neoplasms experienced by the older animals did differ. Neoplasms of the kidney and liver were diagnosed only during 42- and 48-mo periods and lymphoma, histiocytic sarcoma, and Harderian gland neoplasms only during the last 3 monthly periods.

Tumor Incidence

Similar to the effects on longevity, diet had a greater influence than did gender on tumor incidence and tumor number at all ages. Tumors were present in 80.8% of the AL males (SS plus DM) versus 47.3% of the FR males and in 80.2% of the AL females versus 59.6% of the FR females. Thus, despite the fact that both sexes of the FR animals lived about 36% longer (50% survival) than the AL animals (Table II), their cancer incidence over their lifespan was 25 and 17% lower for males and females, respectively (Table IV). Another generality that emerges from inspection of the data in Tables VI and VII is that the prevalence of neoplasms was greater in DM animals than in SS animals at most time intervals. This study underscores that significant differences would be observed in the incidence of fatal tumors in lifetime (longitudinal) studies compared to serial sacrifice (cross-sectional) studies (5). Because animals were routinely removed from the SS pool as moribund (SDM) when clinically apparent tumors became large, those animals were no longer available to the SS pool at the next sacrifice period, accounting for the smaller prevalence of neoplasms in the SS animals (Tables IV and V). A corollary observation, based on the high percentage of tumor-bearing animals in the DM groups, is that neoplasia was the leading cause of morbidity and death in these animals. Further, as depicted in Table V, it was evident that the mean number of tumors per animal increased with age and was greater in AL than FR animals at all times and in both sexes. Table VI also demonstrates the trend toward an increased number of tumors per tumor-bearing mouse with increasing age, for all groups. The greatest increase was in the AL females sacrificed at 36 mo; 13 of the 14 mice examined had a total of 40 neoplasms, an average of 3.1 neoplasms each.

Food restriction appears to have prevented tumor development in a large number of FR mice examined at the 42–48-mo period. In the FR group, 45.2% of the males (67 animals) and 30.6% of the females (34 animals) were still tumor-free at the 42–48-mo interval. In sharp contrast, also emphasizing the protective effect of FR, the AL group had only 7.7% of males and 6% of females still tumor-free at 36 mo, a period that exceeded the 50% survival age for the AL animals (Table IV).

Another perspective of the protracted time-to-tumor and the increased lifespan associated with the FR regimen in the longevity study is provided by the data in Table III. For each of the time intervals and for the total time, the aggregate number of LDM mouse-days on study was calculated by summing the respective age at removal for all animals in the cohort. This number of days was then divided by the total number of neoplasms that were diagnosed in the cohort. The resulting statistic is the average number of "mouse-days at risk" to produce 1 neoplasm, for mice that were removed as dead or moribund during the time period.

NEOPLASMS BY ORGAN SYSTEM

In this section, the tumors are described by organ system. The tumor prevalence (%) was based on the number of animals examined rather than the number of organs examined. This latter number may be

slightly smaller because of occasional missing or autolyzed tissues, especially those of smaller size.

Respiratory System

Female. Alveolar-bronchiolar (lung) neoplasms were present in 21 of the 258 AL mice (8.1%) and in 28 of the 265 FR mice (10.6%). Generally for both diet groups, the prevalence was greater in dead or moribund animals than in their sacrificed cohorts (Table VII). Eight of the 21 neoplasms in the AL mice were carcinomas and 3 of these metastasized. Five of the tumors in the FR mice were carcinomas, with none metastatic.

Male. There were alveolar-bronchiolar neoplasms in 75 of the 245 AL mice (30.6%) and in 39 of the 262 FR mice (14.9%). During the 0–24-mo period, only 2 FR males (4.1%) had an alveolar-bronchiolar neoplasm versus 17 (20.7%) in the AL mice. Thirty-four of the 75 neoplasms in the AL mice were carcinomas and 5 metastasized; none of the 7 carcinomas in the FR mice metastasized. Only 1 other type of neoplasm was associated with the respiratory system, a squamous cell carcinoma in the nasal cavity of a FR male. This animal was removed during the 42-mo period.

Hematopoietic/Lymphoreticular System

These neoplasms were divided into 2 major categories, lymphoma and histiocytic sarcoma. The lymphomas include those diagnosed as mixed cell lymphoma, lymphocytic lymphoma, poorly differentiated lymphoma, and those that could not be subclassified with certainty because of autolysis.

Female. Lymphoma was the most prevalent neoplasm in the AL and FR groups for most time periods (Table VII). This lesion tended to be more frequent among dead and moribund mice than in the sacrifice mice in each time period.

Of 25 histiocytic sarcomas, all occurred in dead or moribund mice, except for 1 in an AL animal sacrificed at 36 mo. The prevalence was similar for both diet groups: 5.8% in AL versus 3.8% in FR (Table VIII). However, they appeared earlier in the AL than FR females.

Male. Lymphoma was diagnosed in male mice in all groups examined except the AL mice sacrificed at 0–24 mo, the FR mice sacrificed at 30 and 36 mo, and the FR dead and moribund mice at 0–24 mo. Only lung neoplasms were diagnosed in more groups. In all, 68 AL mice had lymphoma; only hepatocellular neoplasms (88) and lung neoplasms (75) affected more mice. In both diet groups the total incidence in the dead or moribund mice was greater than their sacrificed cohorts.

Histiocytic sarcomas occurred approximately equally in both diet groups, 6.9% in AL versus 6.5% in FR, but first appeared in the AL group. Similar to the females, only 3 of 34 of these neoplasms were diagnosed in sacrificed mice.

Three other neoplasms of this system were diagnosed in males: 1 granulocytic leukemia in each diet group during the 0–24-mo period and a mast cell neoplasm in a 30-mo dietary restricted LDM.

Digestive System

Female. During the first 2 time periods, hepatocellular neoplasms were present in 19 of the 207 AL mice examined but in none of the 90 FR mice. A total of 31 hepatocellular neoplasms were diagnosed in AL mice, 13 of them carcinomas; 7 metastasized to the lungs. Nine of the 18 liver neoplasms in the FR mice were carcinomas and 1 metastasized to the lungs.

Eight additional neoplasms were diagnosed in the digestive system of AL mice. Three were squamous papillomas of the forestomach, present at the 24-, 36-, and 42-mo periods; 2 others were squamous cell carcinoma of the tongue, which were found at the 24- and 36-mo periods (Table IX). There were single cases of squamous cell carcinoma of the stomach and small intestinal polyp in the 30-mo group and a leiomyosarcoma of the intestine in the 24-mo group. The FR animals had 3 additional digestive system neoplasms: a liver sarcoma at 36 mo and a papilloma and squamous cell carcinoma of the forestomach at the 48-mo period.

Male. Hepatocellular neoplasms were the most common lesions in the AL mice, 88 in 245 mice (Table VIII). Forty of these were carcinomas of which 12 metastasized to the lung. Two hundred sixty-two FR mice had 17 hepatocellular neoplasms; 11 of these were carcinomas, with 3 metastatic to the lung. The total incidence in the dead or moribund mice was greater than in their sacrificed cohort for both diet groups except in the AL mice at 36 mo and the FR mice at 30 mo. No neoplasms occurred in the FR mice during the 0–24-mo period, whereas these periods accounted for 22.7% of the hepatocellular tumors in all AL mice.

Nine additional neoplasms of the digestive system were diagnosed in AL mice. These were a polyp of the large intestine at 18 mo, a liver Ito cell neoplasm at 24 mo, 2 forestomach papillomas at 30 mo, 1 forestomach papilloma and a single gall bladder sarcoma at 36 mo, and 2 squamous cell carcinomas of the forestomach and a carcinoma of the large intestine at 42 mo. Only 4 additional neoplasms were diagnosed in the FR mice. These consisted of 3 polyps of the small intestine at the 36-, 42-, and 48-mo periods and an adenocarcinoma of the glandular stomach at the 42-mo period.

TABLE IX.—Neoplasms of B6C3F1 mice.

SS				SDM	
AL		FR		AL	
Males					
12 Mo (1–456 days)					
	(14)		(14)		(1)
None		None		None	
18 Mo (457–638 days)					
	(14)		(14)		(8)
Liver adenoma	2	None		Liver adenoma	3
Liver carcinoma	2			Liver carcinoma	2
Lung adenoma	1			Lung adenoma	1
				Lymphoma mal	1
				Large intestine polyp	1
24 Mo (639–821 days)					
	(15)		(14)		(19)
Lung adenoma[a]	4	Lymphoma mal	1	Lymphoma mal	5
Liver carcinoma	2	Lung adenoma	1	Lung carcinoma	4
Lung carcinoma	2			Vascular neoplasm	4
Liver adenoma[b]	1			Liver carcinoma	3
Liver ITO	1			Lung adenoma	3
Vascular neoplasm	1			Histiocytic sarcoma	1
				Mesothelioma	1
				Osteosarcoma	1
				Harderian adenoma	1
30 Mo (822–1,003 days)					
	(15)		(15)		(47)
Liver adenoma	4	Liver carcinoma	2	Lymphoma mal	17
Lymphoma mal	3	Liver adenoma	1	Liver carcinoma	12
Lung adenoma	3			Vascular neoplasm	10
Lung carcinoma	3			Liver adenoma[c]	9
Vascular neoplasm	2			Histiocytic sarcoma	7
Liver carcinoma	1			Lung adenoma	6
Adrenal cortex adenoma	1			Lung carcinoma	3
				Harderian adenoma	3
				Stomach papilloma	2
				Abdominal cavity sarcoma	1
				Skin sarcoma	1
36 Mo (1,004–1,186 days)					
	(15)		(15)		(34)
Liver adenoma	7	Lung adenoma	2	Lymphoma mal	12
Lymphoma mal	6	Small intestine polyp	1	Lung adenoma	12
Lung adenoma	3	Kidney adenoma	1	Lung carcinoma	8
Harderian adenoma	2			Liver adenoma	8
Liver carcinoma	1			Liver carcinoma	6
Adrenal cortex adenoma	1			Vascular neoplasm	6
Vascular neoplasm	1			Harderian adenoma	6
				Histiocytic sarcoma	2
				Gall bladder sarcoma	1
				Stomach papilloma	1
42 Mo (1,187–1,368 days)					
	(0)		(19)		(7)
		Lymphoma mal	2	Liver adenoma	3
		Lung adenoma	2	Lung adenoma	3
		Lung carcinoma	1	Lung carcinoma	2
		Harderian adenoma	1	Harderian adenoma	2
		Kidney adenoma	1	Histiocytic sarcoma	2
		Histiocytic sarcoma	1	Vascular neoplasm	2
		Osteosarcoma	1	Chemodectoma	1
				Kidney adenoma	1
				Lymphoma mal	1
				Stomach squamous cell carcinoma	1

TABLE IX.—Extended.

SDM		LDM		
FR		AL		FR
Males				
12 Mo (1–456 days)				
	(1)		(2)	(0)
None		None		None
18 Mo (457–638 days)				
	(0)		(1)	(1)
None		Liver carcinoma	1	Vascular neoplasm 1
24 Mo (639–821 days)				
	(2)		(8)	(3)
Granulocytic leukemia	1	Liver carcinoma	3	Lung adenoma 1
		Lymphoma mal	2	
		Liver adenoma	1	
		Skin fibrosarcoma	1	
		Lung adenoma	1	
		Lung carcinoma	1	
		Harderian adenoma	1	
		Granulocytic leukemia	1	
30 Mo (822–1,003 days)				
	(9)		(21)	(3)
Histiocytic sarcoma	4	Lymphoma mal	9	Mast cell tumor 1
Lymphoma mal	3	Liver carcinoma	5	Lung adenoma 1
Liver adenoma	1	Lung carcinoma	2	
Adrenal pheochromocytoma	1	Lung adenoma	2	
Lung carcinoma	1	Histiocytic sarcoma	2	
Harderian adenoma	1	Islet adenoma	1	
		Liver adenoma	1	
		Lung metastic neoplasm	1	
		Harderian adenoma	1	
		Harderian carcinoma	1	
		Vascular neoplasm	1	
36 Mo (1,004–1,186 days)				
	(20)		(16)	(13)
Lymphoma mal	5	Lymphoma mal	8	Lymphoma mal 5
Lung adenoma	4	Lung carcinoma	5	Histiocytic sarcoma 3
Harderian adenoma	2	Liver adenoma	5	Harderian adenoma 3
Histiocytic sarcoma	2	Vascular neoplasm	5	Lung adenoma 2
Vascular neoplasm	1	Histiocytic sarcoma	3	Testes interstitial cell adenoma 1
		Lung adenoma	2	
		Harderian adenoma	2	
		Liver carcinoma	2	
		Parathyroid adenoma	1	
		Testes interstitial cell adenoma	1	
		Brain oligodendroglioma	1	
42 Mo (1,187–1,368 days)				
	(27)		(8)	(10)
Lymphoma mal	5	Liver adenoma	4	Liver carcinoma 3
Kidney adenoma	5	Lung carcinoma	4	Liver adenoma 2
Adrenal pheochromocytoma	2	Lymphoma mal	4	Kidney adenoma 2
Lung adenoma	2	Vascular neoplasm	1	Stomach adenocarcinoma 1
Lung carcinoma	2	Stomach squamous cell carcinoma	1	Lung adenoma 1
Nose squamous cell carcinoma	1	Adrenal cortex adenoma	1	Small intestine polyp 1
Islet adenoma	1	Large intestine carcinoma	1	Harderian adenoma 1
Harderian adenoma	1			Histiocytic sarcoma 1
Liver adenoma	1			
Kidney adenocarcinoma	1			
Histiocytic sarcoma	1			

TABLE IX.—Continued.

SS				SDM	
AL		FR		AL	
		48 Mo (1,369–1,568 days)			
	(0)		(13)		(0)
		Histiocytic sarcoma	2		
		Lymphoma mal	1		
		Vascular neoplasm	1		
		Lung adenoma	1		
		Harderian adenoma	1		
		Kidney adenoma	1		
		Females			
		12 Mo (1–456 days)			
	(14)		(14)		(1)
Uterus polyp	1	None		None	
		18 Mo (457–638 days)			
	(14)		(14)		(7)
Liver adenoma	1	Harderian adenoma	1	Lymphoma mal	3
Ovary adenoma	1	Ovary teratoma	1	Osteosarcoma	1
Lung adenoma	1			Skin baso sq	1
		24 Mo (639–821 days)			
	(13)		(14)		(32)
Liver adenoma	1	Lung adenoma	1	Lymphoma mal	13
Lymphoma mal	1	Lymphoma mal	1	Liver carcinoma	4
Stomach papilloma	1	Harderian adenoma	1	Histiocytic sarcoma	3
Uterus polyp	1			Liver adenoma	2
Skin sarcoma	1			Uterus leiomyosarcoma	2
				Lung carcinoma	2
				Skin sarcoma	1
				Tongue squamous cell carcinoma	1
				Pituitary adenoma	1
				Thyroid follicular cell adenoma	1
				Ovary granulosa cell tumor	1
				Ovary luteoma	1
				Skin fibrosarcoma	1
				Mammary adenocarcinoma	1
				Uterus polyp	1
				Skeletal muscle rhabdomyosarcoma	1
				Harderian adenoma	1
				Osteosarcoma	1
				Intestine leiomyosarcoma	1
				Mesenteric anaplas neoplasm	1
				Vascular neoplasm	1
		30 Mo (822–1,003 days)			
	(15)		(15)		(73)
Pituitary adenoma	6	Thyroid follicular cell ade-	1	Lymphoma mal	43
Thyroid follicular cell adenoma	4	noma	1	Pituitary adenoma	20
Lymphoma mal	4	Harderian adenoma		Thyroid follicular cell adenoma	12
Uterus polyp	2			Mammary adca	9
Liver adenoma[b]	1			Histiocytic sarcoma	7
Liver carcinoma	1			Lung adenoma	6
Vascular neoplasm	1			Liver carcinoma	5
Stomach squamous cell carcinoma	1			Harderian adenoma	5
Ovary granulosa cell tumor	1			Skin sarcoma	4
Mammary adenocarcinoma	1			Lung carcinoma	4
Lung carcinoma	1			Liver adenoma	3
Harderian adenoma	1			Vascular neoplasm	3
				Skin basosquamous carcinoma	2
				Small intestine polyp	1
				Ovary adenoma	1
				Uterus leiomyoma	1

TABLE IX.—Extended. Continued.

SDM		LDM			
FR		AL		FR	
		48 Mo (1,369–1,568 days)			
	(43)		(0)		(26)
Lung adenoma	11			Kidney adenoma	6
Liver carcinoma	6			Lung adenoma	4
Harderian adenoma	5			Histiocytic sarcoma	3
Lymphoma mal	5			Vascular neoplasm	2
Kidney adenoma	4			Harderian adenoma	2
Adrenal pheochromocytoma	2			Small intestine polyp	1
Vascular neoplasm	2			Islet carcinoma	1
Lung carcinoma	2			Penis squamous cell carcinoma	1
Liver adenoma	1			Lung carcinoma	1
Adrenal cortex adenoma	1			Kidney adenocarcinoma	1
Islet adenoma	1			Lymphoma mal	1
Islet carcinoma	1				
		Females			
		12 Mo (1–456 days)			
	(3)		(1)		(0)
Skin lipo sarcoma	1	None			
		18 Mo (457–638 days)			
	(1)		(2)		(2)
None		Osteosarcoma	1	Ovary teratoma	1
				Lymphoma mal	1
		24 Mo (639–821 days)			
	(3)		(13)		(1)
Thyroid C cell carcinoma	1	Lymphoma mal	8	Lymphoma mal	1
Osteosarcoma	1	Pituitary adenoma	4		
Lung adenoma	1	Lung adenoma	2		
		Thyroid follicular cell adenoma	2		
		Vascular neoplasm	2		
		Mammary adenocarcinoma	1		
		Liver carcinoma	1		
		Uterus polyp	1		
		Uterus leiomyoma	1		
		30 Mo (822–1,003 days)			
	(20)		(22)		(3)
Lymphoma mal	8	Lymphoma mal	17	Uterus leiomyosarcoma	1
Histiocytic sarcoma	3	Pituitary adenoma	4	Skin sarcoma	1
Vascular neoplasm	2	Skin sarcoma	4	Lymphoma mal	1
Skin squamous cell carcinoma	1	Mammary adenocarcinoma	3		
Osteosarcoma	1	Harderian adenoma	2		
Thyroid C cell carcinoma	1	Ovary adenoma	1		
Lung adenoma	1	Uterus polyp	1		
Lung carcinoma	1	Lung adenoma	1		
		Islet adenoma	1		
		Thyroid follicular cell adenoma	1		
		Adrenal pheochromocytoma	1		

TABLE IX.—Continued.

SS				SDM	
AL		FR		AL	
				Uterus leiomyosarcoma	1
				Uterus polyp	1
				Skeletal muscle rhabdomyosarcoma	1
				Harderian adenocarcinoma	1
				Adrenal cortex adenoma	1
				Skeletal muscle sarcoma	1
		36 Mo (1,004–1,186 days)			
	(14)		(15)		(19)
Lymphoma mal[d]	9	Liver adenoma	3	Lymphoma mal	16
Liver adenoma	5	Liver carcinoma	2	Pituitary adenoma	7
Mammary adenocarcinoma	5	Liver sarcoma	1	Thyroid FC adenoma	5
Harderian adenoma	5	Uterus leiomyosarcoma	1	Harderian adenoma	4
Uterus polyp	3	Harderian adenoma	1	Histiocytic sarcoma	3
Pituitary adenoma	3	Lymphoma mal	1	Mammary adenocarcinoma	3
Adrenal pheochromocytoma	2			Ovary granulosa cell tumor	2
Liver carcinoma	1			Uterus leiomyosarcoma	2
Tongue squamous cell carcinoma	1			Liver carcinoma	1
Adrenal cortex adenoma	1			Stomach papilloma	1
Thyroid follicular cell adenoma	1			Adrenal cortex adenoma	1
Ovary granulosa cell tumor	1			Adrenal pheochromocytoma	1
Lung adenoma	1			Ovary luteoma	1
Zymbal's carcinoma	1			Uterus polyp	1
Histiocytic sarcoma[d]	1			Skin tricoep	1
				Lung adenoma	1
		42 Mo (1,187–1,368 days)			
	(0)		(15)		(0)
		Lymphoma mal	2		
		Lung adenoma	2		
		Liver adenoma	1		
		Adrenal cortex adenoma	1		
		Adrenal pheochromocytoma	1		
		Lung carcinoma	1		
		Ovary granulosa cell tumor	1		
		48 Mo (1,369–1,568 days)			
	(0)		(7)		(0)
		Liver adenoma	1		
		Ovary adenoma	1		
		Ovary granulosa cell tumor	1		
		Lung adenoma	1		

[a] Lung adenoma—"4" + 1 that also had a carcinoma.
[b] Liver adenoma—"1" + 1 that also had a carcinoma.
[c] Liver adenoma—"9" + 3 that also had a carcinoma.

Vascular Neoplasms

Female. There were 28 vascular neoplasms: 26 hemangiosarcomas and 2 hemangiomas. Unlike most other neoplasms, these occurred primarily in FR animals, 19 versus 9 in the AL mice. Two of the 9 AL mice and 8 of the 19 FR mice had multicentric lesions. Of the 10 mice with multiple neoplasms, 6 had spleen and liver involvement. The prevalence in the AL mice was constant for the first 3 time periods (3.1, 3.6, and 4.0%) (Table IV). This neoplasm was not found in the 0–24-mo period in

TABLE IX.—Extended. Continued.

SDM		LDM			
FR		AL		FR	
36 Mo (1,004–1,186 days)					
	(34)		(17)		(15)
Lymphoma mal	17	Lymphoma mal	9	Harderian adenoma	3
Vascular neoplasm	5	Pituitary adenoma	8	Vascular neoplasm	3
Lung adenoma	5	Liver adenoma	5	Lymphoma mal	2
Skin sarcoma	3	Thyroid follicular cell adenoma	5	Lung adenoma	2
Histiocytic sarcoma	2	Mammary adca	3	Skin fibrosarcoma	2
Liver carcinoma	1	Vascular neoplasm	2	Skin sarcoma	1
Osteosarcoma	1	Adrenal pheo	2	Adrenal pheochromocytoma	1
Pituitary adenoma	1	Skeletal mus rhabdo	1	Islet adenoma	1
Thyroid FC adenoma	1	Lung adenoma	1	Vagina squamous cell carcinoma	1
Ovary tubular adenoma	1	Lung carcinoma	1	Histiocytic sarcoma	1
Uterus polyp	1	Harderian adenocarcinoma	1		
Skeletal muscle sarcoma	1	Histiocytic sarcoma	1		
Harderian adenoma	1				
42 Mo (1,187–1,368 days)					
	(40)		(1)		(19)
Lymphoma mal	10	Stomach papilloma	1	Lymphoma mal	7
Vascular neoplasm	5	Pituitary adenoma	1	Liver carcinoma	4
Lung adenoma	5			Vascular neoplasm	2
Harderian adenoma	3			Lung adenoma	2
Skin sarcoma	3			Liver adenoma	1
Histiocytic sarcoma	2			Thyroid follicular cell adenoma	1
Thyroid follicular cell adenoma	2			Mammary adenocarcinoma	1
Liver adenoma	2			Skeletal muscle sarcoma	1
Adrenal pheochromocytoma	1			Lung carcinoma	1
Islet carcinoma	1			Harderian adenoma	1
Adrenal cortex adenoma	1			Histiocytic sarcoma	1
Osteosarcoma	1				
Skeletal muscle sarcoma	1				
Lung carcinoma	1				
Kidney adenocarcinoma	1				
Kidney adenoma	1				
48 Mo (1,369–1,568 days)					
	(15)		(0)		(15)
Lymphoma mal	7			Lymphoma mal	4
Liver carcinoma	2			Lung adenoma	2
Vascular neoplasm	2			Liver adenoma	1
Adrenal pheochromocytoma	2			Stomach squamous cell carcinoma	1
Pituitary adenoma	2			Adrenal cortex adenoma	1
Stomach papilloma	1			Adrenal pheochromocytoma	1
Ovary granulosa cell tumor	1			Ovary granulosa cell tumor	1
Harderian adenoma	1			Histiocytic sarcoma	1
Kidney adenoma	1			Kidney adenoma	1
Lung adenoma	1				
Lung carcinoma	1				

the FR mice, but for each of the remaining time periods the prevalence exceeded that in their AL cohorts.

Male. There were 40 vascular neoplasms: 36 hemangiosarcomas and 4 hemangiomas. Thirty-three, including the 4 hemangiomas, were in the AL mice. Of 12 AL mice with multicentric lesions, 11 had liver and spleen involvement. In the 7 FR mice with hemangiosarcomas, 3 had multicentric neoplasms and 1 of these involved the spleen and liver. Vascular neoplasms were one of the 3 neoplasms observed at every time period in AL mice, for both the sacrificed and the dead or moribund groups. The others were lung and liver neoplasms.

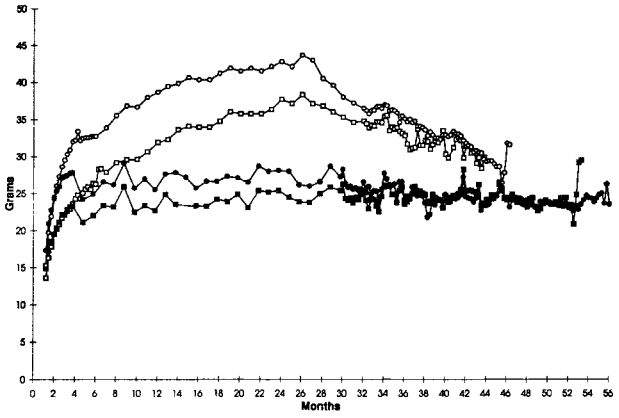

FIG. 1.—Body weight of B6C3F1 mice. Treatment groups are as follows: □ = AL female; ■ = FR female; ○ = AL male; ● = FR male.

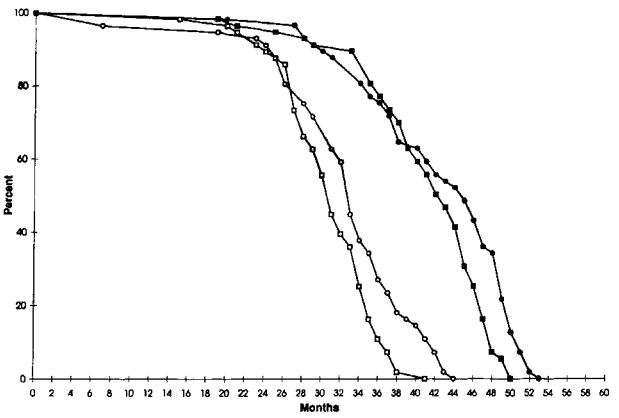

FIG. 2.—Survival curve of B6C3F1 mice. Treatment groups are as follows: □ = AL female; ■ = FR female; ○ = AL male; ● = FR male.

Musculoskeletal System

Female. There were 14 neoplasms of this system: 7 osteosarcomas, 3 rhabdomyosarcomas, and 4 poorly differentiated sarcomas of skeletal muscle. These are listed together in Table VII. Four of the osteosarcomas and 3 sarcomas were in the FR mice and all of the rhabdomyosarcomas were in the AL mice. Seven of these neoplasms metastasized: 4 osteosarcomas, 2 rhabdomyosarcomas, and 1 sarcoma.

Male. Only 2 nonmetastatic osteosarcomas were diagnosed; 1 involved the calvarium of a 24-mo AL mouse and the other a rib of a 42-mo FR mouse.

Integumentary System

Female. There were 27 neoplasms combined in Table VII under Skin and Subcutaneous Tissue. All were diagnosed in dead or moribund mice except for 1 in a sacrificed 24-mo AL mouse. Only 5 neoplasms occurred during the 0–24-mo period; 4 of these were in AL mice. The neoplasms were classified as poorly differentiated sarcomas (18), fibrosarcomas (3), basosquamous carcinomas (3), and single cases of liposarcoma, squamous cell carcinoma, and trichoepithelioma (Skin and Subcutaneous Tissue). The basosquamous cell carcinomas and the trichoepithelioma occurred only in the mice on the AL diet. Of the poorly differentiated sarcomas, 10 were in AL mice and 8 in FR mice. Six neoplasms metastasized: 1 liposarcoma and 3 poorly differentiated sarcomas in FR mice and 3 poorly differentiated sarcomas in AL mice. While the overall frequency was not different in the 2 diet groups, they appeared in higher frequency earlier in the AL than the FR animals.

Male. There were 2 subcutaneous neoplasms in males, both in dead or moribund AL mice. One was a fibrosarcoma at 24 mo and the other a poorly differentiated sarcoma at the 30-mo period, neither metastasized.

URINARY SYSTEM

Kidney

Female. Four animals had a tumor of the tubules of the renal cortex, all in dead or moribund FR mice equally divided between the 42- and 48-mo periods. One neoplasm was a nonmetastasizing adenocarcinoma and 3 were adenomas.

Male. Kidney cortical neoplasms were present in 23 males and all except 1 was on the restricted diet: 3 were in sacrificed and 20 were in dead or moribund mice. Two neoplasms were nonmetastasizing carcinomas and the remainder were adenomas. This neoplasm occurred only once before the 42-mo period; it was in a FR male at 36 mo. This neoplasm had the third highest prevalence rate in FR male mice, 8.4% (Table VIII).

ENDOCRINE SYSTEM

Pituitary

Female. Adenoma of the pars distalis occurred primarily in the AL mice. Fifty-four occurred in the AL and 3 in the FR mice. Most lesions (49 of 54) in the AL mice were found in the last 3 age groups; all of the tumors in the FR mice were observed during these periods (Table VII). This neoplasm was the second most common neoplasm in the longevity AL mice (lymphoma, 60.7%; pituitary adenoma, 30.4%).

Male. No neoplasms of the pituitary were seen in males.

Thyroid

Female. Follicular cell adenomas of the thyroid gland occurred in 31 AL mice and 5 FR mice. With

the exception of 3 cases in AL-fed mice, examined during the 24-mo period, these tumors were detected during the last 3 periods (Table VII). Of the 36 mice with thyroid adenomas, 21 also had pituitary adenomas, only 6 with thyroid adenomas had pituitaries that were normal; the remaining 9 pituitary glands were autolytic or missing.

Two C-cell carcinomas were present in FR mice at the 24- and 30-mo periods.

Male. No thyroid neoplasms were present in males.

Adrenal Gland

Female. Six pheochromocytomas were present in AL mice and 6 in FR mice; all were detected during the last 4 time periods (Table IX). The AL and FR mice each had 3 adenomas of adrenal cortex.

Male. Pheochromocytomas were diagnosed in 5 FR mice and 4 were equally divided in the 42–48-mo periods. There were 3 cortical adenomas observed in AL mice and in 1 FR mouse.

Pancreatic Islet

Female. There were 3 islet neoplasms. One adenoma was diagnosed at the 30-mo period in an AL mouse and 1 adenoma and 1 carcinoma were diagnosed during the 36- and 42-mo periods, respectively, in the FR mice.

Male. One adenoma was present in an AL mouse examined during the 30-mo period and in a FR mouse at 42 mo. One adenoma and 2 carcinomas were present in the FR mice during the last time period.

Parathyroid

A parathyroid adenoma was diagnosed in an AL male at the 36-mo period.

Genital System

Ovary. Neoplasms of the ovary were diagnosed in 10 AL mice and 8 FR mice. Of the 10 in the AL group, there were 5 granulosa cell neoplasms, 3 papillary cystic adenomas, and 2 luteomas. Two of the 8 neoplasms in the FR mice were teratomas, both in the 18-mo group. The other neoplasms, 4 granulosa cell neoplasms, 1 tubular adenoma, and 1 papillary cystic adenoma, were from mice of the last 3 periods (Table IX).

Uterus. Twelve uterine polyps were diagnosed in the AL mice, 1 in the 12-mo group, 3 in the 24-mo group, and 4 each at the 30- and 36-mo periods. Seven smooth muscle neoplasms, 2 leiomyomas, and 5 leiomyosarcomas were also diagnosed in the AL mice. In the FR mice, the diagnosis of uterine polyp was limited to 1 mouse in the 36-mo group and 2 leiomyosarcomas were diagnosed, 1 each from the 30- and 36-mo groups.

Vagina. One squamous cell carcinoma was present in an FR mouse from the 36-mo period.

Mammary. Mammary adenocarcinoma was present in 26 AL and 1 FR female mouse. Only 6 of the 27 neoplasms occurred in sacrificed mice and only 2 (both AL) were in mice younger than the 30-mo group. Ten mice with mammary neoplasms also had pituitary adenomas.

Testis/Penis. There were 3 neoplasms of the male genital system. Two were testicular interstitial cell adenomas; the other was a squamous cell carcinoma of the penis. Both adenomas occurred during the 36-mo period, 1 in each diet group. The carcinoma was in an FR mouse from the 48-mo period.

Other Neoplasms

Female. Two neoplasms are included in this category, 1 a Zymbal's gland carcinoma in an AL mouse from the 36-mo group and the other an anaplastic malignant neoplasm in the abdominal cavity of an AL mouse in the 24-mo group.

Male. Five neoplasms not previously discussed occurred in the AL mice. They were mesothelioma of the abdominal cavity, lung metastasis of an anaplastic neoplasm (the primary neoplasm was not identified), sarcoma of the abdominal cavity, oligodendroglioma of the brain, and a chemodectoma. These occurred in the 24-, 30-, 30-, 36-, and 42-mo groups, respectively.

Harderian

Female. Neoplasms of this gland were observed in all age groups of both diets (except in the single surviving AL mouse in the 42-mo group). There were 33 neoplasms, 18 adenomas and 2 adenocarcinomas in AL mice and 13 adenomas in FR mice. The majority of the neoplasms in both diet groups occurred during the last 2 periods. Twenty-three (70%) of the mice with this neoplasm were dead or moribund.

Male. One 30-mo AL male had an adenocarcinoma of the Harderian gland. Adenomas were present in 35 mice, 18 in the AL group and 17 in the FR group (Table VII). Sixteen of the 17 adenomas in the FR mice were diagnosed in mice from the 36–48-mo periods. Ten of the neoplasms of the AL mice were present in the mice examined at 36 mo. Thirty-two of the 36 mice (89%) with the neoplasm were dead or moribund.

CONCLUSION

The beneficial effects of a calorically restricted diet were evident in this study. The restricted mice of

both sexes lived significantly longer and had a reduced tumor burden compared to their AL cohorts. Based on 50% survival, the FR animals lived approximately 36% longer than the AL animals. However, the total number of neoplasms developed over the lifespan of the FR males was 167 versus 325 in their AL cohorts. Females had a similar increase in their 50% survival and a 50% reduction in tumors over the lifespan, 210 in FR versus 401 in AL. The suppression of neoplasia appears to have lasted throughout the entire lifespan of the FR males and females since 41 and 31%, respectively, of the males and females examined during the 42–48-mo period were free of neoplasms. The extended longevity of the FR mice of both sexes can be explained partially by the reduced incidence and delay of occurrence of lymphoma. Similar reductions in occurrence of lung and liver neoplasms contributed to the longevity of the males. In the FR females, the occurrence of these latter 2 tumors was also delayed, although their lifetime incidence was not appreciably different from the AL cohort. Nonneoplastic progressive degenerative diseases (a subject of a future report) were similar in incidence in both groups but were more severe in the AL mice, especially the females. These diseases also contributed to the earlier deaths of AL mice.

The major tumors (summarized in Table VIII) were affected by dietary restriction in 4 primary ways: (a) FR had minor impact on the lifetime incidence of some tumors. (It did affect the time of their appearance). In both sexes the incidence of the neoplasms of the Harderian gland, musculoskeletal system (except rhabdomyosarcomas in the females), skin/subcutis, and the incidence of histiocytic sarcoma, were only slightly reduced compared to their incidence in the AL mice. (b) The increased longevity permitted the opportunity for neoplasia of the renal tubules, a rare kidney tumor, to develop in the FR males. Only 1 of 27 of these neoplasms was diagnosed before 42 mo of age. Also, FR appears to have enabled a larger number of vascular and alveolar-bronchiolar neoplasms to develop in the longer-lived FR females than in their AL cohorts, because the incidence of these entities increased in aged mice. (c) Food restriction had a strong suppressant effect on hormone-dependent neoplasms. Neoplasms of the pituitary, thyroid, and mammary gland occurred in high incidence in the AL females but seldom in FR females and not at all in males of either diet group. (d) Dietary restriction appeared to have delayed the onset and reduced the incidence of lymphomas in both sexes and alveolar-bronchiolar tumors and liver neoplasms in the males. The liver neoplasms in the FR females were delayed, but the overall incidence was not appreciably different from their AL cohorts.

The serially sacrificed mice of both sexes and diet groups had far fewer neoplasms than their age-matched dead or moribund cohorts because the SS groups were monitored daily to remove mice that were moribund or dead. The primary reason for their removal was neoplasia and usually "fatal" tumors.

SUMMARY

The incidence of various neoplasms described in this report for *ad libitum*–fed animals dying spontaneously is similar to previous reports in untreated B6C3F1 mice of comparable age (2, 4). We are unable to compare the tumor incidences reported here for the scheduled sacrifice groups or the dietary-restricted groups with previous literature because no comparably designed experiment has been reported. Despite this, there are 3 broad generalizations common to our dietary-restricted mice and FR mice of other genotypes maintained under different experimental conditions. These generalizations are (a) lifespan is increased, (b) tumor incidence is reduced, and (c) tumor onset is delayed.

REFERENCES

1. Bronson RT and Lipman RD (1991). Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth Dev. Aging* 55: 169–184.
2. Goodman DG, Boorman GA, and Strandburg JD (1985). Selection and use of B6C3F1 mouse and F344 rat for carcinogenicity studies. In: *Handbook of Carcinogenicity Testing,* HA Milman and EK Weisburger (eds). Noyes Publications, Park Ridge, Nevada, pp. 282–325.
3. Meitas J (1990). Aging: Hypothalamic catecholamines, neuroendocrine–immune interaction, and dietary restriction. *Soc. Exp. Biol. Med.* 195: 304–311.
4. Tamano S, Hagiwara A, Shibata M, Kurata Y, Fukushima S, and Ito N (1988). Spontaneous tumors in aging (C57BL/6N C3H/HeN)F_1 (B6C3F_1) mice. *Toxicol. Pathol.* 16(3): 321–326.
5. Weindruch R and Walford RL (1988). *The Retardation of Aging and Disease by Dietary Restriction.* Charles C Thomas, Springfield, Illinois.
6. Witt WM, Brand CD, Attwood MS, and Soave OA (1989). A Nationally supported study on caloric restriction of rodents. *Lab. Animal* 18: 37–43.