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Diet, Overfeeding, and Moderate Dietary Restriction in Control Sprague-Dawley Rats: I. Effects on Spontaneous Neoplasms*

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ABSTRACT

This study was designed to compare the effects of *ad libitum* (AL) overfeeding and moderate dietary restriction (DR) of two different diets on Sprague-Dawley (SD) rat 2-yr survival and the development of spontaneous neoplasms. SD rats were fed Purina Rodent Chow 5002 or a modified Rodent Chow 5002-9 containing lower protein, fat, metabolizable energy and increased fiber by AL or by DR at 65% of the AL amount by measurement or time (6.5 hr). At 106 wk, rats fed the 5002-9 diet AL did not have significantly improved survival over rats fed the 5002 diet AL. The 5002 diet fed DR by time (6.5 hr) improved survival for males but not females. Only DR by measurement of both diets resulted in lower mortality for both sexes. The most common cause of death in rats of both sexes fed either diet AL was pituitary tumors followed by mammary gland tumors in females and renal and cardiovascular disease in males. The overall tumor incidence by 106 wk was remarkably similar between AL and DR groups. However, compared to the 5002 AL group, a decrease in the age-adjusted (Peto analysis) incidence of pituitary adenoma was observed in all other male groups. This effect was noted in the female DR by measurement groups only. For males, compared to the 5002 AL group, a decrease in the age-adjusted incidence of pancreatic islet carcinoma was observed in the DR by measurement groups only. In females, compared to the 5002 AL group, the only other difference in tumor incidence was the mammary gland tumors, which showed a significant decrease in the age-adjusted tumor incidence or multiplicity in the 5002-9 AL, 5002-9 DR, and 5002 DR groups. Additional analyses of mammary gland tumors showed growth time (time from initial palpation until death), tumor doubling time, and tumor volume were generally not statistically significantly different between AL and DR groups, although AL females could sustain larger tumor volumes. Compared to the 5002 AL group, there were no other significant differences in the age-adjusted incidence of any other tumor site in animals fed a modified diet or subjected to moderate DR of either diet. The conclusion from this study is that moderate DR delays death due to fatal cardiovascular or renal degenerative disease and spontaneous tumors, particularly those of the pituitary and mammary gland. However, moderate DR appears only to delay the time of onset, but not the progression, of these spontaneous tumors whether measured by age-adjusted incidence, growth time, tumor doubling time, or the time between initial detection and death.

Keywords. Survival; caloric restriction; spontaneous neoplasms; aging

INTRODUCTION

Laboratory rat survival in 2-yr carcinogenicity studies has been declining over the past 3 decades throughout the pharmaceutical and chemical industry (8, 21–27, 33–35, 48, 53, 59–61). This decline has been seen in all rat strains, including the Sprague-Dawley (SD) and the Fischer rat (F-344), the most commonly used rats in toxicity and carcinogenicity studies (21, 34, 59). This decline in survival has caused some regulatory agencies to question the adequacy of exposure in rats on carcinogenicity studies that result in less than 50% survival, or 25 animals alive per group at the end of the 2-yr period (8, 22, 35). While both genetic and environmental factors are involved, rat survival can be improved

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by simple dietary restriction (DR) (14, 15, 40, 41, 43, 45, 75, 81).

We have observed a significant correlation between average daily food consumption and 2-yr survival in control, *ad libitum* (AL) fed SD rats. Data from 58 control groups of SD rats from 2-yr carcinogenicity studies initiated during the 1980s indicated considerable inter- and intralaboratory variability in the range of average survival, body weight, and food consumption from study to study (26, 27). Two-year survival of male controls from this rat stock ranged from 7% to 73% and daily food consumption of the same diet ranged from 21 to 32 g/day (27). These data show that food consumption and survival are tightly correlated but very variable from laboratory to laboratory. Therefore, any attempt to modify AL food consumption by DR must be done with an appreciation of the wide variability in average food consumption between different laboratories (27). Also, the animals in the above-mentioned studies consuming amounts of feed in the upper range (i.e., males eating 32–33 g/day) are grossly overfed with predictable effects of the early development and progression of degenerative disease of the kidney and heart and tumors of the pituitary and mammary gland leading to premature death.

This paper presents data showing the association between AL overfeeding and poor survival, the early onset of serious renal and cardiovascular degenerative disease, and potentially fatal tumors, particularly those of the pituitary and mammary gland. The results of this study indicate that moderate DR of SD rats delays the time of onset of pituitary and mammary gland tumors, but not their overall incidence nor their progression if the animals are maintained for a significant portion of their life span as is the case in a 24-mo carcinogenicity study.

Materials and Methods

Animals. Three hundred fifty male and 350 female SD rats (Crl:CD®(SD)BR) were obtained from Charles River Laboratories, Raleigh, NC. The animals were 36 days of age at the initiation of the study with the males weighing 115–175 g and the females weighing 91–156 g. The rats were individually housed in stainless steel wire cages in environmentally controlled clean air rooms with a 12-hr light/dark cycle. The animals were identified by tattoos and assigned to the 5 different treatment groups described below using a balanced random allocation scheme. Rats selected for the 52-wk interim necropsy and the 106-wk terminal necropsy and also for organ weights and minipump implantment were assigned by a stratified random allocation procedure as follows: For each sex and diet group the rats were ordered by body weight from the lowest to the highest weight. They were then divided into 10 strata by body weight and 1 rat from each strata was randomly chosen for the given necropsy with a second backup animal selected in the event the primary animal did not survive until the necropsy date. This procedure was chosen to optimize the probability that a truly representative sample of animals from each dietary regimen would be sampled from the necropsy.

Diet and Dietary Regimen. The experimental groups contained 70 rats/sex/group and were designed to compare two different diets as well as moderate DR. The diets and DRs were as follows:

a) Purina Certified Rodent Chow 5002 fed AL (5002 AL) as pellets (approximately 24 g/day for females and 33 g/day for males). This diet contains approximately 21.4% protein, 5.7% fat, 4.1% crude fiber, and has a calculated metabolizable energy value of 3.07 kcal/g.

b) Certified Rodent Chow 5002 fed AL for approximately 6.5 hr/day during the light cycle (5002 6.5 hr DR).

c) Purina Certified Rodent Chow given in measured amounts daily (5002 DR) at approximately 65% of our adult SD rat AL food consumption (approximately 16 g/day for females and 21.5 g/day for males).

d) Purina Certified Rodent Chow 5002-9 fed AL (5002-9 AL) as extruded pellets (this diet contains approximately 13.6% protein, 4.6% fat, 15.7% crude fiber, and has a calculated metabolizable energy value of 2.36 kcal/g).

e) Purina Certified Rodent Chow 5002-9 fed in measured amounts (5002-9 DR) to provide approximately the same caloric intake as animals fed under regimen c (approximately 20.8 g/day for females and 28.8 g/day for males).

Necropsy, Histopathology, and Tumor Studies. All rats dying spontaneously, killed moribund, or surviving until scheduled necropsy underwent a complete necropsy examination. All rats, including those selected for bromodeoxyuridine (BrdU) minipump implantation, special tissue biochemistry, and carcass analysis were weighed, deeply anesthetized by ether inhalation, and killed by exsanguination. The minipumps were removed; terminal body weights and organ weights were taken. Organs containing masses were not weighed. Each animal underwent a complete gross necropsy and numerous tissues were sampled, including all gross lesions. The tissue samples were routinely fixed in 10% neutral buffered formalin (testes fixed in Bouin's solution), and routine histological sections of paraplast-embedded tissues were stained with hematoxylin and eosin from all rats and included salivary gland, stomach, small intestine, large intestine, liver, pancreas, adrenals, pituitary, thyroid and parathyroid,

kidneys, urinary bladder, ovaries, uterus, testes and epididymides, prostate, skin, mammary gland, heart, lung, spleen, lymph nodes, thymus, bone and bone marrow, skeletal muscle, brain, spinal cord, sciatic nerve, eyes with optic nerve, Harderian gland, and any gross lesions.

Because mammary gland tumors can be palpated and measured when the animals are alive, an analysis of growth rate was performed on histologically confirmed mammary gland tumors of all types on the female rats in this study. The 5 treatment groups were compared for total mammary gland tumor volume, estimated tumor growth rate, and tumor growth time (time from initial palpation to death from all causes including terminal necropsy). Each group was compared to the 5002 AL group. Because survival differed among groups and because mammary tumor growth rate was observed to slow over time, tumor-bearing females were stratified into 2 groups based on the time from initial palpation until death and included those females bearing tumors from 0 to 25 wk after palpation and those animals bearing tumors beyond 26 wk after palpation. In addition, the time to 50% or 25% prevalence of mammary gland tumors by palpation was determined by the Kaplan–Meier curves for time to tumor. Growth time was defined to be the time in weeks from initial palpation until death (either scheduled or unscheduled). If tumor palpation occurred in the same week as death or if the tumor was not detected until necropsy, the growth time was defined for convenience to be 0.5 wk. Tumor volume was defined as the total volume by measurement of all mammary gland tumors present at necropsy and confirmed by histology. Average tumor volume was defined as the geometric mean of total tumor volume for each animal with a mammary tumor. The tumor doubling time was calculated from the slope of the curve relating growth time to tumor volume, either over growth times of 0–25 wk or over 25+ wk.

Statistics. Mortality curves over the 106-wk study were summarized with life tables as described by Kaplan and Meier (23) and were compared using the log-rank test (55). Tumor incidences were compared using the method of Peto et al (56), including an adjustment for age and context of tumor observation (observed prior to death, observed at necropsy and deemed the cause of death, observed at necropsy but not deemed the cause of death).

Mammary gland tumor data on female rats including time to 25% and 50% prevalence, tumor growth time, tumor volume, and tumor doubling time were analyzed with the aid of the Kaplan–Meier curves (log rank test) with scheduled sacrifice treated as censoring. Time to 25% and 50% prevalence was also analyzed by Kaplan–Meier curves for time to tumor rather than time to death. Analyses of these data were by log rank test. Analysis of variance was used to compare multiplicity of mammary tumors among groups, restricted to tumor-bearing females. These data are summarized in Table II and show a p-value in the last column of the table as a global test for any differences among dietary groups. If statistically significant, then a pairwise comparison of each group with the 5002 AL group was done (71). These pairwise comparisons are reported using an * in the body of Table II.

In order to quantify the sensitivity, or power, of carcinogenicity studies to detect a treatment effect, 4 variables must be specified (47): a) the number of animals studied; b) the distribution over time of death in tumor-free animals; c) the distribution over time of tumor onset; and d) for nonpalpable tumors, the effect of tumors on subsequent survival. For nonpalpable tumors, it is not possible to observe all these variables unless multiple interim necropsies are done. For palpable tumors, however, time of tumor onset can be approximated by time of initial palpation. Power calculations are most accurate for common tumors, because they provide more data for estimating the distribution of tumor onset time. Therefore, we restricted our attention to mammary tumors (of all types) in females and calculated the power of a carcinogenicity study under either AL feeding or moderate DR.

Power was calculated in 3 steps. First, data from this study were used to estimate the distribution of time to mammary tumor and time to death in controls under either the 5002 AL or 5002 DR diets. Second, these distributions were used to specify a statistical model for mammary tumors in carcinogenicity studies. For simplicity, we considered a study with 1 control and 1 treated group. Like the present study, the model used 70 females per group. Third, 500 carcinogenicity studies were generated from the model by computer simulation, analyzed by Peto's test, and the percentage with statistically significant results recorded. For the power calculations the significance level $p = 0.01$ was used, a level often used in carcinogenicity studies for common tumors. Percentages were reported ± 1 standard error.

Results

Effects of Diet, Overfeeding (AL), and Moderate DR on Survival and Cause of Death

The mortality results obtained from analysis of the Kaplan–Meier survival curves have been reported in detail elsewhere (26). These data are summarized here and in Table I for comparison to the spontaneous carcinogenicity results and the degenerative lesions discussed below. Briefly, analysis of survival to 104 wk with adjustment for the 52-wk

TABLE I.—Summary of survival and incidence of primary neoplasms and lesions.

Group	5002 AL		5002 6.5 hr DR		5002 DR		5002-9 AL		5002-9 DR	
	F	M	F	M	F	M	F	M	F	M
% Survival	36	67	42[ns]	57[s]	62[s]	74[s]	24[ns]	32[s]	81[s]	75[s]
No. necropsied	70	70	70	70	70	70	70	70	70	70
Nephropathy	39 (1)	64 (11)	14	41	3	19	44 (1)	55 (2)	6	18
Cardiomyopathy	13	31 (5)	15 (1)	32	2	11	18	25	3	12
With neoplasms	61	50	58	51	58	49	61	51	47	34
With benign neoplasms	61	48	55	40	57	40	61	45	40	30
With malignant neoplasms	15	14	20	22	22	15	25	20	17	9
Pituitary										
Focal hyperplasia	9	15	8	19	9	17	7	17	12	20
Adenoma	50 (21)	40 (20)	49 (22)	32** (13)	47** (15)	28** (7)	55 (29)	37** (14)	31** (4)	19** (4)
Carcinoma	1 (1)	0		0	3 (3)	0	0	0	1	0
Mammary gland										
Adenocarcinoma	9 (2)	0	15 (4)	0	10 (1)	0	17 (5)	0	6*	0
Adenoma	6	1	6	0	6	0	8	1	0**	0
Fibroadenoma	32 (8)	2	20*	3	25* (2)	0*	18** (3)	2	12**	1 (1)
Fibroma	1	0	1 (1)	0	0	1	1	0	0	0
Pancreas										
Acinar adenoma	0	1	0	0	0	0	0	0	0	0
Islet, adenoma	3	4	1	2	1	5	3	3	1	3
Islet, carcinoma	0	4	1	7	0	1*	0	5	2	0**
Histiocytic sarcoma	0	0	0	0	0	1 (1)	0	0	0	0
Adrenal										
Cortex, adenocarcinoma	0	1	0	0	1	0	0	0	1	0
Cortex, adenoma	1	0	1	0	1	2	1	2	0	1
Benign pheochromocytoma	0	3	0	2	0	0*	1	2	1	0
Malignant pheochromocytoma	0	1	0	0	3	2	0	0	0	1
Benign schwannoma	0	0	1	0	0	0	0	0	0	0
Liver										
Bile duct, adenoma	0	0	0	0	0	1	0	0	0	0
Bile duct, carcinoma	0	1	0	0	0	0	0	0	0	0
Hepatocellular adenoma	1	2	1	1	1	3	1	2	2	1
Hepatocellular carcinoma	1	4	0	2	0	2	1	3 (1)	0	1
Thyroid										
Parafollicular cell adenoma	3	3	4	7	2	4	4	4	4	3
Parafollicular cell carcinoma	0	0	0	0	2	0	1	1	1	0
Follicular cell adenoma	0	3	1	1	0	0	0	0	0	0
Parathyroid										
Adenoma	0	0	0	0	0	0	0	0	0	1

TABLE I.—Continued.

Group	5002 AL		5002 6.5 hr DR		5002 DR		5002-9 AL		5002-9 DR	
	F	M	F	M	F	M	F	M	F	M
Ovary										
Benign granulosa cell tumor	3	0	0	0	0	0	1	0	0	0
Benign luteoma	0	0	0	0	0	0	0	0	1	0
Malignant theca cell tumor	0	0	0	0	0	0	0	0	1	0
Uterus										
Endometrial adenocarcinoma	1	0	0	0	0	0	0	0	0	0
Hemangioma	0	0	0	0	0	0	1	0	0	0
Leiomyoma	0	0	0	0	1	0	0	0	0	0
Leiomyosarcoma	0	0	0	0	0	0	0	0	1	0
Endometrial stromal polyp	1	0	1	0	1	0	3	0	4	0
Endometrial stromal sarcoma	0	0	0	0	1	0	0	0	0	0
Vagina										
Squamous cell papilloma	0	0	0	0	0	0	1	0	0	0
Squamous cell carcinoma	0	0	1	0	0	0	0	0	0	0
Testis										
Interstitial cell adenoma	0	1	0	6	0	1	0	5	0	1
Malignant mesothelioma	0	3	0	1	0	1	0	0	0	0
Prostate										
Adenoma	0	0	0	0	0	1	0	1	0	1
Skin										
Benign basal cell tumor	0	3	0	1	1	0	0	0	0	0
Malignant basal cell tumor	0	0	0	1	0	0	0	0	0	0
Fibroma	1	2	0	2	0	1	1	4	0	3
Fibrosarcoma	1 (1)	1	0	4 (3)	0	2 (2)	1 (1)	2 (1)	0	0
Histiocytic sarcoma	0	0	0	0	0	1 (1)	1 (1)	1 (1)	0	0
Keratoacanthoma	0	1	0	1	2	2	1	5	0	1
Lipoma	1	3 (1)	0	0	0	1	0	0	0	2
Papilloma	0	0	0	1	0	2	0	1	0	0
Malignant schwannoma	0	0	0	0	0	1	1	0	0	1 (1)
Kidney										
Tubular adenoma	0	0	1	0	1	0	0	1	0	1
Liposarcoma	2	0	0	0	0	0	0	0	0	0
Urinary bladder										
Transitional epithelium, carcinoma	0	0	0	0	0	0	0	0	1 (1)	0
Stomach										
Nonglandular mucosa, squamous cell carcinoma	0	0	0	0	0	0	0	2	0	0

TABLE I.—Continued.

Group	5002 AL		5002 6.5 hr DR		5002 DR		5002-9 AL		5002-9 DR	
	F	M	F	M	F	M	F	M	F	M
Small intestine										
Adenocarcinoma	0	0	0	0	0	1	0	0	0	0
Adenoma	0	0	0	0	0	0	0	0	0	1
Leiomyoma	0	0	0	0	1	0	0	0	0	0
Large intestine										
Adenoma	0	0	0	0	0	1	0	1	0	0
Peritoneum										
Fibrosarcoma	1	0	0	0	0	0	0	0	0	0
Nose										
Osteosarcoma	0	1 (1)	0	0	0	0	0	0	0	0
Squamous cell carcinoma	0	0	0	1 (1)	0	0	1 (1)	0	0	0
Lung										
Bronchiolar adenoma	0	0	0	1	0	0	1	0	0	0
Heart										
Endocardium, malignant schwannoma	0	0	0	1	0	0	0	0	0	0
Bone										
Vertebral column, osteosarcoma	0	0	0	0	0	0	0	1 (1)	0	0
Skeletal muscle										
Histiocytic sarcoma	0	0	0	0	0	1	0	0	1	0
Rhabdomyosarcoma	0	0	0	1 (1)	0	0	0	0	0	0
Brain										
Astrocytoma	0	0	0	1 (1)	1	1	0	2 (1)	0	1
Granular cell tumor	1 (1)	0	0	1	0	0	0	0	0	0
Oligodendroglioma	0	0	0	0	0	0	0	0	1 (1)	0

TABLE I.—Continued.

Group	5002 AL		5002 6.5 hr DR		5002 DR		5002-9 AL		5002-9 DR	
	F	M	F	M	F	M	F	M	F	M
Eye										
Harderian gland, adenoma	0	0	0	0	1	0	0	0	0	0
Melanoma	0	1	0	0	0	1 (1)	0	0	0	0
Squamous cell carcinoma	0	0	0	0	0	0	1 (1)	1	0	0
Ear										
Zymbal's gland, adenoma	0	1	0	0	0	0	0	0	0	0
Zymbal's gland, carcinoma	0	0	0	2 (1)	0	0	0	0	0	2
Neurofibroma	0	0	1	0	0	0	0	0	0	0
Spleen										
Hemangiosarcoma	1 (1)	0	0	1 (1)	0	0	0	2 (1)	0	1 (1)
Histiocytic sarcoma	0	1	0	0	0	0	0	0	0	0
Thymus										
Lymphoma	1	0	0	0	2	0	0	0	0	1
Benign thymoma	0	0	0	0	0	0	1	0	0	0
Malignant thymoma	0	0	0	0	0	0	0	1	0	0
Primary site undetermined										
Histiocytic sarcoma	0	0	0	1 (1)	1 (1)	0	0	0	2 (1)	0
Liposarcoma	0	1 (1)	0	0	0	0	0	0	0	0
Lymphoma	2 (1)	1	2 (2)	1 (1)	1 (1)	1 (1)	1 (1)	1 (1)	1	1 (1)

Data: n = number of rats with a given diagnosis; (n) = number of rats whose cause of death was a given diagnosis.
^{ns} $p > 0.10$ compared with the 5002 AL group (Kaplan–Meier).
$^{\wedge}$ $p \leq 0.001$ compared with the 5002 AL group (Kaplan–Meier).
* $p < 0.05$ tumor incidence compared to 5002 AL group, not adjusted for multiplicity of statistical tests.
** $p < 0.05$ tumor incidence compared to 5002 AL group, adjusted for multiplicity of statistical tests.

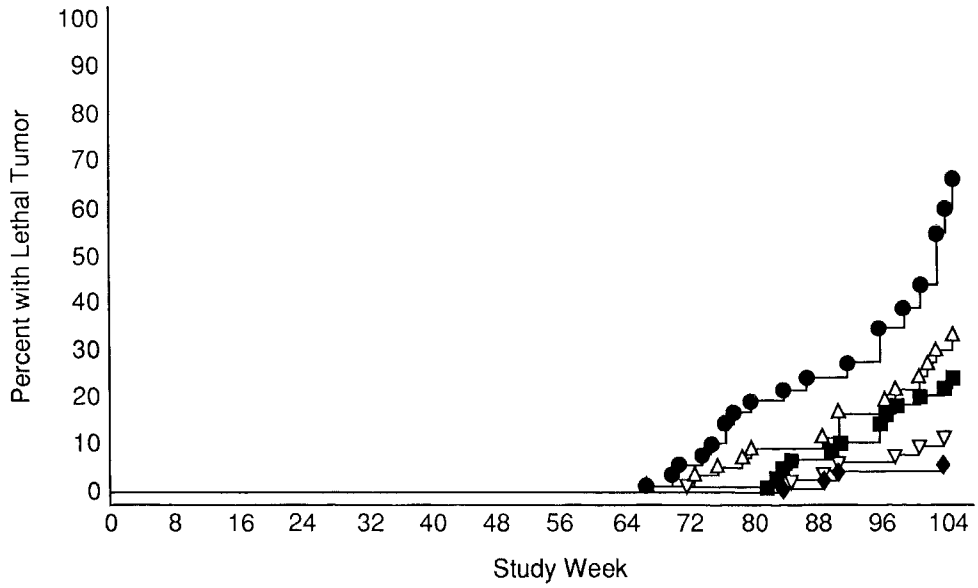

FIG. 1.—The cumulative percentage of male SD rats with lethal pituitary tumors. ● = 5002 AL, ■ = 5002 6.5 hr DR, ▽ = 5002 DR, △ = 5002-9 AL, ◆ = 5002-9 DR.

interim necropsy showed that only measured DR of the 5002 DR and the 5002-9 DR groups resulted in lower mortality for both males and females compared to the 5002 AL group. The time-restricted group, 5002 6.5 hr DR, had better survival in males but the survival was not statistically significant in 5002 6.5 hr DR females. The 5002-9 AL group did not have an improved survival in females and had less than 50% survival in males. Only measured DR of either diet resulted in improved survival in both sexes (26).

The cause of death or reason for euthanasia was determined by morphologic diagnosis. The most common cause of death in all groups of AL and DR rats fed either diet was pituitary tumors that resulted in large intracranial masses that produced fatal brain compression. Death from pituitary tumors in the 5002 AL and 5002-9 AL groups of both sexes and the 5002 6.5 hr DR females was an earlier event than in the DR groups, suggesting an earlier onset of these potentially fatal tumors (Figs. 1 and 2). Mammary gland tumors were a common cause of death in the 5002 AL females and 5002-9 AL females. Benign or malignant mammary gland tumors usually resulted in death by euthanasia when a large mammary gland mass had ulcerated and resulted in a moribund condition of the individual. Metastatic mammary gland adenocarcinomas were rare and noted only in 1 5002 6.5 hr DR female and 1 5002-9 AL female. After pituitary tumors in both sexes and mammary gland tumors in females, the next most common causes of death were severe renal disease and cardiovascular disease in the AL fed males, but these conditions were not observed as a cause of death in the DR rats of either sex fed either diet. The other causes of death observed in this study reflected a general distribution among the common tumors and degenerative conditions seen in this rat stock (11, 36, 48, 79). The largest number of undetermined deaths was seen in the 5002 AL males. In general, both sexes of the AL fed groups given either diet had an earlier onset and/or more severe lesions and tumors than the 5002 DR or the 5002-9 DR fed rats. The only unusual cause of death was chronic colitis and fecal impaction leading to chronic colonic dilatation secondary to the consumption of the 5002-9 high fiber extruded diet. Six males and 3 females in the 5002-9 AL group and 1 female in the 5002-9 DR group died or were euthanized from the effects of the high fiber content of this diet on their large intestines.

Effects on Spontaneous Neoplasms

The spontaneous tumor incidence data from this 106-wk carcinogenicity study are summarized in Table I. Morphologic changes observed in all of the tissues examined in all the groups reflect common neoplastic, proliferative, or degenerative processes commonly seen in control SD rats during the course of a 2-yr carcinogenicity study in this laboratory. The overall incidence of neoplasia, both benign and malignant, was remarkably similar among all groups. However, both sexes of the 5002 AL and 5002-9 AL groups and females in the 5002 6.5 hr DR groups tended to have an earlier onset and larger neoplasms and these tumors were more likely to be contributing factors to early mortality. In contrast, both sexes of the 5002 DR, 5002-9 DR groups, and the males of

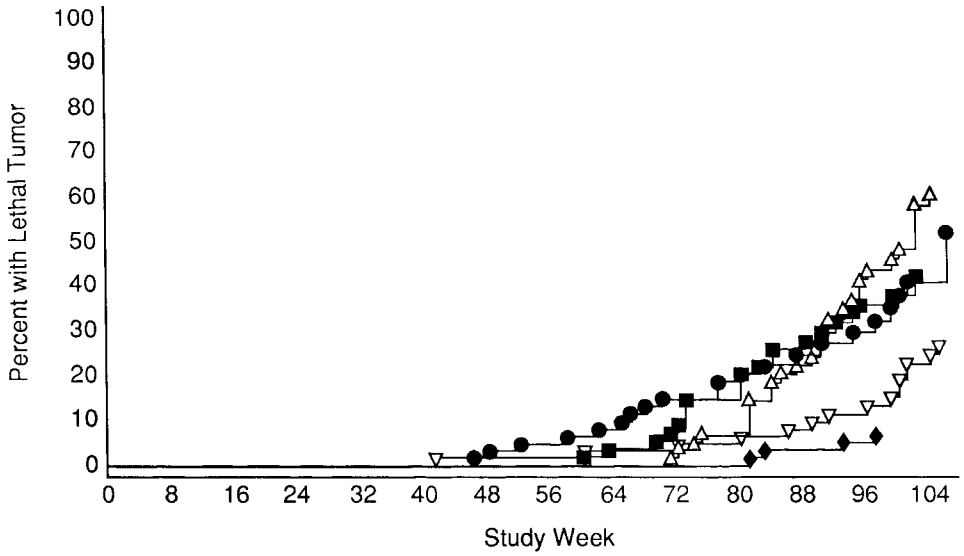

FIG. 2.—The cumulative percentage of female SD rats with lethal pituitary tumors. ● = 5002 AL, ■ = 5002 6.5 hr DR, ▽ = 5002 DR, △ = 5002-9 AL, ◆ = 5002-9 DR.

the 5002 6.5 hr DR group, while having a similar overall incidence of neoplasia had a later tumor onset and tended to have smaller tumors that were found incidentally rather than resulting in early mortality.

For each sex separately, the statistical methodology employed involved a pairwise comparison of the 5002 AL groups with the 4 other treatment groups. This methodology incorporated context-of-observation for nonpalpable tumors (distinguishing lethal and nonlethal tumors), time to tumor detection for palpable tumors, time to death without tumor, and the use of a discrete permutation test for tumor sites with few tumor-bearing rats. In addition, when applying statistical analysis simultaneously to many tumor sites, an adjustment for multiplicity of statistical tests was done separately for each sex. Statistical analyses for each dietary regime compared with 5002 AL are given in Table I. The p-values are based on 1-sided Peto tests that are adjusted for age of death, time to tumor detection, and tumor lethality. Tumor types yielding $p \leq 0.05$ before adjustment for multiplicity of statistical tests are marked with an * in Table I; if statistical significance remained after adjustment for multiplicity, the tumor type is marked **. Unless otherwise stated, p-values for tumorigenicity in this report are adjusted for multiplicity of statistical tests.

A decrease in the age-adjusted incidence of pituitary adenoma was observed in every male treatment group compared with the 5002 AL males ($p < 0.05$). In females, a decrease in the age-adjusted incidence of pituitary adenoma was observed only in the measured DR groups (5002 DR and 5002-9 DR, $p < 0.05$).

In males, a statistically significant ($p < 0.018$) decrease in the age-adjusted incidence of pancreatic islet carcinoma was observed in the 5002-9 DR group. Borderline statistical significance was observed in the 5002 DR group ($p = 0.063$). The overall incidence of pancreatic islet cell adenoma and carcinoma when combined was similar in these groups compared to the 5002 AL group and the differences in pancreatic islet cell carcinoma appeared to reflect a greater progression in the growth (size) of this common spontaneous nonmetastasizing endocrine tumor of the pancreatic islets.

For females, the only other tumor types showing statistically significant ($p < 0.05$) changes in incidence were mammary gland tumors. A decrease in the age-adjusted incidence of mammary gland fibroadenoma ($p < 0.05$) was seen in both the 5002-9 AL and 5002-9 DR groups and approached statistical significance in both the 5002 6.5 hr DR ($p = 0.056$) and the 5002 DR ($p = 0.063$) groups. In addition, the 5002-9 DR females had a lower incidence of mammary gland adenoma ($p = 0.038$) and adenocarcinoma ($p = 0.042$).

The incidence table and the age-adjusted tumor incidence analysis (Peto analysis) do not indicate fully how dietary restriction alters mammary tumor growth rate. Because mammary tumors can be palpated and measured when the animals are alive, an analysis of growth rate is possible with mammary tumors of all types, the most commonly palpable tumor in the SD rat. Mammary tumors in female

TABLE II.—Mammary tumor volume and estimated doubling time.

	5002 AL	5002 6.5 hr DR	5002 DR	5002-9 AL	5002-9 DR	*p*-value
Survival to 104 wk (%)	36	42	62***	24	81***	<0.001
No. females with tumor/total	39/70	34/70	33/70	33/70	17/70***	0.003
No. tumors/female with tumor	1.9	1.7	1.2**	1.5	1.0***	<0.001
Time to 25% prevalence (wk)	62	69	73**	77*	97***	<0.001
Time to 50% prevalence (wk)	85	85	101**	93*	>105***	<0.001
0–25 Wk after palpation						
No. females with tumor	26	24	20	26	15	n.a.
Mean growth time (wk)	11	13	12	7.6	7.9	n.a.
Doubling time (wk)	3.1	3.1	4.7	4.2	4.8	0.479
Average tumor volume (cm^3)	29	25	19	18	12	0.451
26+ Wk after palpation						
No. females with tumor	13	10	13	6[a]	2	n.a.
Mean growth time (wk)	37	36	35	30	33	n.a.
Doubling time (wk)	12	26	5.2	—[b]	—[b]	0.489
Average tumor volume (cm^3)	308	86	68	144	86	0.071

[a] Rat 91843 excluded: tumor volume of 0.25 cm^3 at death 40 wk after palpation.
[b] Negative or zero estimated growth rate; doubling time not defined.
n.a.: Not analyzed (see text).
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ compared with Purina 5002 AL group.

rats from the 5 treatment groups are compared in Table II for total mammary gland tumor volume, estimated tumor growth rate, and tumor growth time (time from initial palpation to death or necropsy, including terminal necropsy). Each group was compared to the 5002 AL group. Tumor volume was defined as the total volume by measurement of all mammary gland tumors present at necropsy and confirmed histologically. Growth time was defined as the number of weeks from first palpation of a mammary gland tumor until death from any cause including terminal necropsy. Average tumor volume was defined as the geometric mean of the total tumor volume for each animal with a mammary tumor. The doubling time or time for the tumor to double in size was calculated. Because survival differed among groups and because mammary tumor growth rate was observed to slow over time, tumor-bearing females were stratified into 2 groups based on time from initial palpation until death to include those with tumors from 0 to 25 wk after palpation and those bearing tumors for greater than 26 wk after palpation. The results are given in Table II, and the last column in the table (*p*-value) is a global test for any differences among dietary groups. If statistically significant, then a pairwise comparison for each group with the 5002 AL group was done. These pairwise comparisons are reported using asterisks in the body of Table II.

For each dietary group the tumor doubling time is estimated from the slope of the curve relating tumor growth time to tumor volume either over growth times of 0–25 wk or over 26+ wk. Because of individual animal variability, there was some imprecision in estimating doubling times. Two difficulties are noted in the table for the 26+ growth time stratum. First, for the 5002-9 AL group, the estimated slope was negative, which would suggest the tumors were shrinking in size. This is not the case, but simply reflects the scatter in the data set. Second, for the 5002-9 DR group, there were only 2 rats in this stratum and both happened to have the same growth time, so a slope could not be estimated.

The time to 25% or 50% prevalence analyzed by the Kaplan–Meier curves indicate the time to tumor for the DR groups was significantly longer than that for either of the AL groups, with the exception of the 5002 6.5-hr DR group. Thus, although most groups had similar numbers of tumor-bearing animals, females in both of the measured DR groups had a later initial palpation time on average. For example, the time to 50% prevalence for the 5002 AL group was 85 wk, while for the 5002 DR group it was 101 wk, a difference of 16 wk that was highly significant. In addition, females in the 5002 DR and 5002-9 DR groups had fewer tumors per tumor-bearing animal, even after adjustment for survival differences. However, for females with less than 26 wk of tumor growth time, no significant differences were observed between any AL or DR group, either in mean growth time, estimated doubling time, or average tumor volume. Females with tumors for 26 wk or more before death had, likewise, no difference from DR groups on average growth time or doubling time.

Using the survival and mammary tumor palpation data in females from the 5002 AL and 5002 DR groups, the power of a carcinogenicity study was estimated by computer simulation. Both AL and

DR had approximately a 1% false-positive rate ($p = 0.62$). If a hypothetical treatment doubled the risk of mammary tumor onset throughout the study, the estimated power was 80% ± 2% under either AL or DR ($p = 0.87$). Because the risk of mammary tumor onset was generally less each week under DR compared with AL, doubling the risk under DR was a smaller increase compared with AL. If the effect of hypothetical treatment on mammary tumor onset occurred primarily late in the study, then the estimated power under AL was 59% ± 2%, significantly less ($p = 0.001$) compared with 69% ± 2% under DR. This is not surprising, because the 5002 DR females had significantly better survival than 5002 AL females.

Analysis of all other tumor sites, including nonpalpable, nonlethal tumors and tumor sites at which 10 or fewer tumor-bearing animals were observed, indicated no statistically significant age-adjusted incidence of tumors in any diet or dietary restricted group compared to the 5002 AL group (Table I).

Discussion

Many factors are known to be causative determinants of chronic degenerative disease and cancer, as shown by studies of human pathology and epidemiology, as well as from research in experimental animals. The role of multiple factors in disease can be not only additive, but also interactive, including synergistic as well as inhibitory interactions. Dietary factors associated with overeating and decreased activity patterns have been shown to be the second major nongenetic external factors that contribute to human death in the United States after tobacco use (17, 38, 46, 51). Dietary factors in humans have been associated with cardiovascular disease, including coronary artery disease, stroke and high blood pressure, cancers of the colon, uterus, ovary, breast and prostate, and diabetes mellitus (17, 38, 46, 51). Therefore, it is not surprising that sedentary laboratory rodents, when overfed, suffer from an early onset of degenerative disease and the early development of diet-related tumors (14, 15, 25–27, 40, 45, 60, 61, 80). Survival and food consumption data on control SD rats from 58 2-yr carcinogenicity studies showed a significant relationship between percent survival and average food consumption for SD rats fed the same feed (26). The greater the daily food consumption, the greater the likelihood of decreased survival by 104 wk. These and other data show that food consumption, body weight, and survival are tightly correlated, but have considerable variability between and within different laboratories using the same rat stock and identical diets (26). Nevertheless, it appears it is the amount of feed provided per day per animal that is critical in the long-term survival of SD rats rather than a given percentage of extremely variable AL levels reported from these different laboratories. The level of DR practiced in this study does not fall outside of the range of AL feeding in other laboratories and demonstrates that the AL food consumption in our laboratory results in gross overfeeding of the animals with predictable adverse results on their health and survival (26). It is noteworthy that only rodents are maintained by AL feeding, whereas, other laboratory animals, such as dogs and primates, are fed measured amounts of feed, and it is considered a poor veterinary practice to do otherwise.

DR, as practiced in this study, had no adverse effects on the overall long-term health or lesion incidence of the DR animals. The evaluation of the various dietary regimens indicates that the 5002 AL animals had a higher incidence, earlier onset, and/or greater severity of the common degenerative and proliferative lesions seen in rats of this stock on 2-yr carcinogenicity studies. However, the overall incidence of benign and malignant neoplasia and lesions observed in both the AL and DR groups were within the control range of tumors and degenerative lesions observed in 2-yr carcinogenicity studies with this SD stock (11, 36, 46, 51 and Merck Research Laboratories database). While many of the neoplasms occur at a similar incidence, the tumors observed in the AL fed rats were generally of earlier onset, larger, more severe, and more likely to be contributing factors to their early death. In contrast, several of the degenerative diseases, particularly chronic renal disease and cardiovascular disease, showed clear differences in onset, incidence, and severity between the AL and DR groups, with serious renal and cardiovascular disease frequently identified as a contributing factor to the early death of many of the 5002 AL and 5002-9 AL males.

It is well established that DR through reduced energy intake will reduce the incidence of spontaneous and induced neoplasms depending on the rodent species or strain and on the particular neoplastic lesion (1, 3, 15, 16, 25–30, 40, 57–65, 69, 70, 74, 79, 81). This influence on neoplastic disease appears to be partially responsible for the increased survival observed under DR. However, the extent that DR affects tumor progression following the onset of spontaneous neoplastic lesions remains to be examined for most tumor types as does the role of DR on life extension. While it is reported that DR of a given AL caloric intake or diet decreased the incidence of certain spontaneous neoplasms in rodents (58, 60–62, 74, 81), our studies and those of others indicate this is not true for all types of tumors when an evaluation is made at either the time of spontaneous death or when rats are maintained for

a significant portion of their life span as in the case of a 24-mo carcinogenicity study (26, 39, 45, 69, 70).

In lifetime studies of F-344 rats fed AL or DR at 60% of the AL amount, the percentage of rats with neoplastic disease at the time of spontaneous death was greater in DR groups than AL fed rats (39, 70). However, this finding may be due to the much greater age at which DR rats can live. When theoretical survival curves were generated on these studies based on all neoplastic disease or specific neoplastic disease (leukemia/lymphoma) as the cause of death, the results of those analyses showed that the effects of DR delayed death due to neoplastic disease in general (70, 71). The main influence of DR on F-344 male rats indicates energy restriction significantly influences the onset rate of spontaneous tumors. Energy restriction appears to delay the occurrence of death due to leukemia/lymphoma in F-344 male rats, but DR had this action by delaying the onset of leukemia/lymphoma and not by decreasing the progression and severity of the disease after onset occurs (69). Restriction of protein or fat without decreased energy intake was without this effect (70). These observations are consistent with our SD rat data that indicate the time of onset for pituitary and mammary gland tumors is delayed by moderate dietary restriction, but not the growth rate or progression after these tumors develop.

Pituitary tumors were the most common cause of death in SD rats fed either diet AL or DR. The overall incidence of these neoplasms was very similar between most of the AL and DR groups. However, compared to the 5002 AL group, the age-adjusted incidence indicated a significantly decreased incidence in all other male groups and in the measured DR female groups. Plots of mortality due to pituitary tumors suggested an earlier onset of these potentially lethal tumors in the AL fed rats (Figs. 1 and 2). Data reported from the 52-wk interim necropsy indicated the 5002 AL and 5002-9 AL rats of both sexes and the 5002 6.5 hr DR females had larger pituitaries, higher BrdU % labeling index (LI) of normal cells in the *pars distalis,* and the highest incidence of focal pituitary cell hyperplasias and adenomas compared to the other groups (26). The earlier onset of these pituitary lesions correlated with the progression of pituitary tumors during the second year when they became chronic, space-occupying, intracranial lesions that produced chronic ventral midbrain compression, degeneration, hydrocephalus, and eventual death from intracranial pressure.

In addition to contributing to the rats' death by intracranial pressure, pituitary tumors are also prolactin secreting, which contributes to the pathogenesis of mammary gland tumors. The development of prolactin-secreting pituitary tumors in both sexes of aging rats is associated with a reduction of hypothalamic dopamine activity. The escape from hypothalamic control leads to lactotroph cell hyperplasia and a high incidence of prolactin cell tumors in old rats (49, 52, 66, 67, 73, 77, 78). The decrease in secretion of dopamine and other catecholamines by hypothalamic neurons also lowers hypothalamic release of gonadotropin-releasing hormone, growth hormone-releasing hormone, and thyrotropin-releasing hormone. These result not only in increased prolactin secretion by the pituitary, but also reduced pituitary secretion of gonadotropic hormones, growth hormone, and thyrotropic hormone with expected multiple endocrine alterations in target cell responses (9, 49, 50, 67). The results of this study are consistent with studies that indicate AL overfeeding accelerates reduction of hypothalamic dopamine activity, increased pituitary prolactin release, and results in the early development of potentially fatal pituitary and mammary gland tumors in the rat (49, 52, 73). In contrast, moderate DR has a major protective effect on the hypothalmus and has been shown to prevent age-related decreases in hypothalamic dopaminergic activity, without reducing the responsiveness of pituitary to hypothalamic hormones or the target endocrine glands to pituitary hormones (9, 49, 50).

Because pituitary tumors are prolactin secreting, it is not surprising that the early onset of these tumors in the AL females was associated with the early development of mammary gland tumors. Results from the 52-wk interim necropsy show the 5002 AL and 5002-9 AL females were the only groups with fibroadenomas and they had the highest incidence of galactoceles at that time (26). Except for the 5002-9 DR females, the final overall incidence of benign and malignant mammary gland tumors was similar between all groups. However, a clear delay in the time of onset was evident in measured DR groups. The time of initial palpation and the time to 25% or 50% tumor prevalence was significantly longer in both measured DR groups compared to their AL counterparts. The mean growth time, estimated doubling time, and average tumor volume were not different in any group of females bearing mammary tumors for less than 26 wk. In AL females with mammary tumors palpated 26 wk or more before death, larger tumor volumes were seen than in the DR females, but growth time and doubling time were not statistically different. The difference in adjusted tumor volumes could arise from a difference in tumor volumes between AL and DR groups at initial palpation or from a difference in tumor growth rate during the follow-up period. A difference in

tumor volume at initial palpation was not seen and tumor volumes and growth rate were similar during the 25 wk following initial palpation. The differences in tumor volume seen in the females with tumors for 26 wk or more after palpation are most consistent with the hypothesis that mammary tumor growth after palpation is similar in the AL and DR females, but that AL females are able to develop larger tumor volumes in certain individuals due to their larger body size and possibly their larger mammary gland mass. These results show that moderate DR by caloric restriction of either diet caused a delay in the time of onset of mammary tumors, but did not affect mammary tumor growth rate after initial palpation.

The 5002-DR and 5002-9 DR males had a decreased age-adjusted incidence of pancreatic islet cell carcinoma, but a higher incidence of islet cell hyperplasia than the AL males. These data and results from the 52-wk interim necropsy (26) indicate an earlier onset and progression in the growth (size) of this nonmetastatic islet cell tumor in AL males. These findings are consistent with results from other studies that indicate DR fed rats have decreased serum insulin levels and insulin-like growth factor I/somatomedin C levels (29, 30, 62, 65). These data also correlate with studies that show DR fed rats have lower plasma glucose and insulin levels, but are able to maintain glucose utilization as well as AL fed rats (42, 44). These and other studies indicate glucose effectiveness and/or insulin sensitivity are increased by moderate DR and that AL overfeeding can result in many features typically seen in non-insulin-dependent diabetes and are consistent with the glycation theory of aging (10, 42, 44, 75). These data also suggest the complex interactions that the early development of these endocrine and metabolic changes may have on the development and progression of these spontaneous tumors of the pituitary, mammary gland, and pancreatic islets in AL overfed rats.

Analysis of the age-adjusted tumor incidence of all other tumor sites, including nonpalpable, nonlethal tumors indicated no significant difference in tumor incidence with either diet or dietary restricted group compared to the 5002-AL group. For each tumor type, there was remarkable similarity in the number of tumor-bearing animals across groups. The main effects on spontaneous carcinogenesis after adjustment for survival are the DR groups show a later death from lethal pituitary tumors (both sexes) and the DR females show a later average onset time for mammary tumors compared to the 5002 AL. Moreover, even with tumors of the pituitary, mammary gland, and pancreatic islets that show an age-adjusted difference in incidence, the observed incidence of these tumors in the DR animals was not outside of the range of historical controls in our laboratory or those reported for this SD stock by other laboratories (11, 36, 48, 79 and Merck Research Laboratories database).

Delaying the time of tumor onset or even decreasing the incidence at a given time point should not decrease the ability of a 24-mo bioassay conducted by moderate dietary restriction to detect a compound-related carcinogenic effect. The moderate dietary restriction of the 5002 diet in this study, while still in the range of AL food consumption in other laboratories, resulted in good survival and increased the exposure time animals would have to a compound. In this study, the average time on study for 5002 AL males was 80.1 wk, while 5002 DR males were on study for 99.6 wk (26). This difference means the 5002 DR males would have about 19.5 wk (5 mo) of additional exposure to a test compound. This increased exposure time is highly significant and would increase the statistical sensitivity of the bioassay to detect a treatment-related event (26).

When the statistical power for detecting a 2-fold increase in risk of mammary tumors was calculated by computer simulation using the observed survival and palpation distributions for females, both regimens yielded similar statistical power (i.e., 80% for 5002 AL and 80% for 5002 DR, $p = 0.87$). However, if a hypothetical compound caused a 2-fold increased risk of mammary tumors only after a long exposure to a compound, then the estimated power was only 59% for the 5002 AL females, but 69% for the 5002 DR females ($p = 0.001$). Thus, compared with AL groups, moderate DR delays the onset time for mammary tumors, but with the improved survival, results in comparable or improved statistical power to detect a treatment-related effect.

Studies of the effect of dietary and caloric restriction on induced tumorigenesis by known direct- and indirect-acting carcinogens do show a lower incidence and later onset at a given time point depending on the level of restriction (1, 16, 28–30, 57, 62, 64, 65, 80). However, many of these experiments were terminated at 5–12 mo. Nevertheless, these studies do detect treatment-related tumors in the caloric-restricted animals that clearly distinguishes them from untreated controls (1, 28–30, 64, 65, 74, 80). Thus, the ability of these bioassays to detect a carcinogenic response at even early time points meets the working definition of adequate statistical sensitivity or power. Compared to AL overfed rats, a larger number of moderately DR fed rats will live to 24 mo, and significantly more animals will be exposed to the test compound over the 2-yr period increasing the bioassay's sensitivity to detect treat-

ment effects (26). The declining survival seen in AL overfed rats lowers statistical sensitivity, especially for late occurring tumors.

A number of mechanisms have been postulated to account for the reduction of induced and spontaneous tumorigenesis by dietary or caloric restriction (15, 16, 27, 40, 50, 62, 63, 75, 80, 81). These include a general reduction in growth of normal and neoplastic tissues due to reduced growth-promoting hormones such as insulin, somatomedin-C, growth hormone, prolactin, and other mammotrophic hormones and increases in growth-inhibiting adrenal corticoids. These endocrine mechanisms are very likely operative in the moderately DR-fed SD rat considering the effects on pituitary and mammary gland tumorigenesis seen in this study.

The effects of dietary restriction in the reduction of oxidative damage to DNA and other macromolecules (proteins, enzymes, and lipid peroxidation) due to modulation of free radical production and damage and maintaining normal scavenging enzyme activities and glutathione levels support the free radical theory of aging and have been shown to be operative in many studies including the present study (10, 19, 26, 31, 32, 37, 40, 42–44, 54, 72, 75, 76). Also, reduced glucose and insulin levels in DR rats have lent support to the glycation theory of aging that proposes glucose as a mediator of aging and tumorigenesis by nonenzymatic glycation of proteins and nucleic acids. In overfed AL rats glycation is similar to that occurring in diabetes mellitus (10, 40, 42–44, 75). These and other mechanisms are postulated to result in a reduction of cell division, DNA synthesis, DNA adduct formation, and alterations in DNA repair in given target tissues of dietary restricted animals (3, 14, 15, 40, 64, 80, 81). In addition, alteration of the metabolism of carcinogens, other xenobiotics, and steroid hormones in the liver, due to enzyme changes related to adrenal glucocorticoid responses, have been proposed to be operative in DR fed rats (14, 15, 54, 64, 81). However, in our studies of moderate DR we have been unable to demonstrate significant differences from AL fed rats in basal or induced hepatic enzymes involved in biotransformation including phase I and phase II systems (25, 27). Moreover, little consideration has been given to the fact that all these mechanisms, if operative, are enhanced and accelerated by AL overfeeding.

The wide variability seen between different laboratories' AL food consumption, body weights, survival, and control tumor incidence with the SD rat and other rat stocks and strains demonstrates that AL food intake is one of the greatest uncontrolled and underestimated variables remaining in rodent toxicity and carcinogenicity studies (26, 27). The multiple negative effects of AL overfeeding can mask the specific effects of a given compound on carcinogenicity. The variability in food intake with its chronic multiple effects on many tissues may result in numerous interactive effects that may not only be additive, but possibly inhibitory if they mask a late treatment effect due to early mortality (26, 35).

The need to control major nongenotoxic determinants in a carcinogenicity study is seen in the effects of chronic infections enhancing the development of human and experimental animal cancers (2, 4, 5, 13, 18, 20). In studies of respiratory tract carcinogenesis in rats treated with the systemic carcinogen, *N*- nitrosoheptamethyleneimine, progressively higher incidences of peripheral lung carcinomas were seen in germ-free (17%), specific pathogen-free (37%), and conventionally *Mycoplasma*-infected rats with chronic pneumonia (83%) (68). These and other murine infections that are known to affect carcinogenesis are now strictly controlled by the use of specific pathogen-free animals, microbial monitoring, and current husbandry practices. Moreover, it is generally accepted that nongenotoxic nutritional, mechanical, and toxicological injuries to target tissues that result in cell death, increased cell proliferation and regeneration, and that have dose-related thresholds, safety margins, or established mechanistic relationships should be considered enhancing factors that need to be carefully controlled. Examples include mechanical wounding and phorbol esters in skin (2), bile acid injury and infections in the colon (4), various toxic, infectious, and resection injury in the liver (13, 20), saccharin and mechanical injury in the urinary bladder (12), mechanical wounding, toxic injury, and vitamin A deficiency in respiratory tract carcinogenesis (24), foreign body tumorigenesis (6), and even wounding related to development of plant tumors (7). Therefore, the variable and frequently excessive food consumption presently reported in AL fed rat carcinogenicity studies (25–27) is a major uncontrolled variable in these critical chronic bioassays with serious implications for their use as models of human risk assessment.

The results of this study underline the complex multisystemic mechanisms induced by overfeeding and underline the healthful action of moderate DR on SD rat longevity, spontaneous carcinogenesis, and age-related proliferative and degenerative lesions. A number of major hypotheses have been proposed for the beneficial effects of dietary restriction, but many have been ruled out by recent basic studies (14, 40, 45, 75, 81). However, it is apparent that the fundamental mechanisms induced by overfeeding or moderate dietary restriction do involve the means by which rodents utilize fuel in more or

less damaging ways over the course of their life span. Most of the basic work in dietary restriction supports hypotheses of reducing the adverse metabolic effects of glucose and oxygen, both of which are potential toxic processes (10, 19, 31, 32, 37, 40–42, 44, 45, 72, 75, 76). Results from our study do support the most widely held basic hypotheses that caloric restriction acts by modulating the characteristics but not the rate of fuel use in a way to prevent the long-term damage or preserve the protective molecular mechanisms against oxidative damage or glycation resulting from such fuel use (40, 44, 45, 75). In contrast, our present methods of overfeeding rodents AL apparently accelerates these aging processes that result in lower survival and the early onset and increased severity of age-related degenerative diseases and diet-related endocrine tumors. The data in this study are consistent with observations of others over the past decades that reducing dietary energy intake by caloric restriction will increase life span, retard age-related senescence and degeneration, and delay or prevent the appearance of age-related diseases and tumors that are accelerated by AL overfeeding. Some evolutionary biologists have noted that the AL fed laboratory rodent may really be a case of pathological aging, while the supposedly postponed aging of the food-restricted rodent represents a more normal aging pattern (63). Nevertheless, the beneficial effects of this moderate level of DR in improving longevity, preventing the development of chronic degenerative disease, and delaying the onset of diet-related endocrine tumors will result in the animals being exposed to test compounds for a longer period of time and this improves the sensitivity of the bioassay to detect compound-specific chronic toxicity and carcinogenicity. This standardized, controlled method of moderate DR provides a given amount of food that is still within the range of current AL food consumption (26) and results in a 2-yr overall incidence of spontaneous neoplasms that is in the range reported in control SD rats of this source (11, 36, 48, 79, 81 and Merck Research Laboratories database). While both genetic and environmental interactions are involved, it is clear that low rat survival is closely correlated with AL overfeeding and can be improved by simple dietary caloric restriction. Moderate DR does not adversely affect the rats' health, physiology, metabolic profile, or spontaneous tumor incidence and, thus, improves the rat carcinogenicity bioassay for the evaluation of human safety.

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