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Diet and the Suitability of the Male Fischer 344 Rat as a Model for Aging Research

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There has been concern about the suitability of the male Fischer 344 (F344) rat as a model for aging research because of the high prevalence of a single disease, severe nephropathy, at advanced ages which confounds the interpretation of an aging study. In a publication from our laboratory, Iwasaki et al. (1988) reported that replacing the casein in our standard semisynthetic diet with soy protein markedly decreases the progression of nephropathy with advancing age in ad libitum fed male F344 rats. In the present study, it is shown that replacing the casein with lactalbumin does not decrease the occurrence of severe nephropathy in ad libitum fed rats. It is also shown that dietary restriction (DR) studies can be effectively executed in the male F344 rat when soy protein is the source of dietary protein. It is further shown that when the energy intake of the rats fed soy protein-containing diets was reduced to 60% of the ad libitum intake, almost one-third of the rats died with an absence of severe morphologic lesions, that is, lesions which contribute to the death of the rat. It is concluded that the male F344 rat is an excellent model for aging research when soy protein is the source of dietary protein; no single disease process was found to be primarily responsible for death with such a diet.

FOR many years, the male Fischer 344 (F344) rat has served as a major animal model for aging research (Masoro, 1990). Recently, concerns have been expressed about its appropriateness for use in aging studies [summarized in an editorial by Weindruch and Masoro (1991)]. The major issue is the occurrence in the male F344 rat of severe nephropathy in a high percentage of the animals at advanced ages (Coleman et al., 1977). It is viewed that the occurrence of a single severe disease in most of the animals confounds the interpretation of an aging study.

It is important that many genotypes be studied in order to generalize findings and, therefore, the use of new strains — in particular F_1 hybrids — is to be encouraged. Nevertheless, because such a large body of data has been collected on the male F344 rat, it is critically important that work be continued with this model under conditions that permit interpretation of the findings within an aging context. Indeed, the nephropathy problem in this strain can be eliminated by restricting the energy intake of the rats (Maeda et al., 1985), a manipulation commonly referred to as dietary restriction (DR). Unfortunately, many investigators wish to use ad libitum fed (AL) rats rather than DR rats as the standard model, thereby eliminating this solution of the problem. However, work carried out in our laboratory (Iwasaki et al., 1988) has shown that manipulation of the protein source of the diet can to a great extent eliminate this problem even in ad libitum fed rats. Specifically, replacing the casein as the protein source in our standard semisynthetic diet with soy protein greatly reduced the occurrence of severe grades of nephropathy, i.e., grades of severity associated with the elevation of serum creatinine and blood urea nitrogen levels. For example, of the 10 rats randomly selected for sacrifice at 24 months of age, none had severe or very severe lesions; of the 7 rats sacrificed at 27 months of age, 2 had severe lesions and none had very severe lesions. Moreover, of the 70 rats that died spontaneously (median age of 28 months), only 7% had very severe grade lesions.

Clearly, the soy protein-containing diet makes it possible to execute life span studies with male F344 rats without severe nephropathy being a serious confounding problem. However, two questions remained to be addressed before broadly recommending the use of this dietary regimen for aging studies with this rat model. One is whether casein is a uniquely nephropathogenic dietary protein. The other is whether DR studies can be successfully executed with soy protein as the dietary protein source. This article reports the findings of a study designed to address these questions.

Materials and Methods

Rat maintenance and dietary procedures. — Male F344 rats were purchased as weanlings (26 to 30 days of age) from the Kingston, NY, facility of the Charles River Laboratories. They were maintained in the specific pathogen-free condition, singly housed in a barrier facility operated as described previously (Yu et al., 1985). A 12:12 h light cycle was used. Sentinel rats were sacrificed on receipt and at 6-month intervals for monitoring of viral antibodies (Sendai, Reo-3, GD-VII, PVM, KRU, H-I, SDA, LCM and Adeno) and for Mycoplasma Antibodies in serum samples sent to Microbiological Associates (Bethesda, MD). All tests were negative.

For the first two weeks, all rats were fed ad libitum a semisynthetic diet (Diet A), the detailed composition of which was described previously (Bertrand et al., 1980). At 6 weeks of age, the rats were divided into four dietary groups, Groups A, L, B, and S. Group A was fed Diet A ad libitum. Group L was fed ad libitum Diet L, which has the same

composition as Diet A except for replacement of casein with lactalbumin and the modification of the mineral mix to provide Group L rats with a similar mineral intake as the rats in Group A. Group B was fed Diet B at about 60% the energy intake of Group A; the composition of Diet B, which is similar to Diet A, was described previously (Bertrand et al., 1980). Group S was fed Diet S at about 60% the energy intake of Group A; the composition of Diet S is similar to Diet B except for the use of soy protein instead of casein and the modification of the mineral mix to provide Group S with a mineral intake similar to Group B.

The amount of food eaten by each rat in Groups A and L was measured as described previously (Yu et al., 1985). The daily energy intake was calculated. Each rat in Groups B and S received a daily allotment of food about 1 hour before the start of the dark phase of the light cycle. The usual bedding material was replaced with antibiotic-treated cage boards (Shepherd Specialty Papers, Kalamazoo, MI) beneath the wire-mesh cage floor in order to permit the easy detection of food spillage. Body weight of each rat was measured at 2-week intervals.

Procedures following spontaneous death of the rats. — All rats were inspected at least twice daily (from 0700 to 0800 and from 1500 to 1600 h). Dead rats were removed from the cage and either necropsied immediately or refrigerated for a brief period. Autolysis was almost never severe enough to prevent grading of lesions.

The following organs and tissues were excised and fixed in 10% formalin: brain, pituitary gland, heart, lungs, trachea, aorta, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, prostate, testes, epididymis, seminal vesicles, thyroid gland, adrenal glands, parathyroid glands, psoas and thigh muscles, sternum, femur, and vertebrae. The tissues were examined histologically as described previously (Iwasaki et al., 1988). In addition, any other tissue with a gross lesion was examined histologically.

Nephropathy was graded in the order of increasing severity: Grade 0 (no lesions), Grade 1, Grade 2, Grade 3, Grade 4, and Grade E. The grading system has been described previously (Yu et al., 1982). Photomicrographs of each grade of lesion have been published (Maeda et al., 1985). Elevated levels of blood urea nitrogen and serum creatinine were observed only in rats with Grade 4 or Grade E lesions.

Cardiomyopathy was graded in the order of increasing severity: Grade 0 (no lesions), Grade 1, Grade 2, Grade 3. The grading system and a photomicrograph of Grade 3 lesions were presented in the report of Maeda et al. (1985).

Hepatic bile duct hyperplasia and hepatic fatty change were graded in order of increasing severity: Grade 0 (no lesions), Grade 1, Grade 2, Grade 3. A description of the grading systems and photomicrographs of Grade 3 of both lesions were presented in the report of Maeda and colleagues.

The prevalence and severity of leukemia/lymphoma were determined by the procedure of Peto et al. (1980); categories 1, 2, 3, and 4 were considered to be increasing grades of severity.

The prevalence and/or severity of pituitary hyperplasia and adenoma were determined as follows: Hyperplastic nodules less than 1 mm in diameter were classified as hyperplasia; lesions greater than 1 mm in diameter were classified as adenoma; Grades 1, 2, 3, and 4 of increasing severity were based on the increasing likelihood that the tumor played a role in the death of the rat.

Statistical analysis. — The survival curves were estimated using product limit estimates, and curves were compared using Wilcoxon test (Gross and Clark, 1975). The median and 10th percentile survival times of the dietary groups were compared using the quantile test (Conover, 1971). Food intake and body weight were compared for dietary groups and age differences by analysis of variance (Snedecor and Cochran, 1967).

The total frequency of a lesion or grade of lesion was analyzed with a chi-square test for the rats that died spontaneously (Siegel, 1956). When the expected frequencies were too small for the chi-square test, the data were analyzed with Fisher's exact test for 2×2 tables (Siegel, 1956).

RESULTS

Food intake and body weight. — Data on food intake are reported in Figure 1. The rats in Group A and Group L had a similar intake of energy. Group B and Group S rats consumed throughout the life span almost all of the daily allotment of food and therefore had a daily energy intake approximately 60% of that of the Group A and L rats.

The body weights for Group A and Group L rats were similar (Figure 2). The body weights of the Group B and Group S rats were similar and over most of the life span significantly below those of the Group A and Group L rats.

Longevity. — The survival curves for the rats in Groups A, L, B, and S are presented in Figure 3. The curves for Group A and Group L rats did not differ significantly. The curves for Group B and Group S rats also did not differ but were significantly different from the survival curves for Group A and Group L rats ($p < .001$).

The median length of life, the age of the 10th percentile survivors, and the maximum length of life are reported in Table 1. Rats of Group A and Group L did not differ significantly in median length of life or in age of 10th percentile survivors nor did the rats of Group B differ from those in Group S in these regards. However, the median length of life and age of the 10th percentile survivors were significantly greater for the rats in Groups B and S than for the animals in Groups A and L ($p < .001$).

Chronic nephropathy. — Severe (Grade 4) or very severe (Grade E) chronic nephropathy was present at the time of spontaneous death in 50% or more of the rats in Groups A and L (Table 2) but in 5% or less of the rats of Groups B and S. Moreover, more than 80% of the rats in Groups B and S had no lesions (Grade 0) or minimal lesions (Grade 1) at the time of spontaneous death compared to 10% or less of the rats in Groups A and L. Although the difference in severity was highly significant when comparing rats on energy-restricted

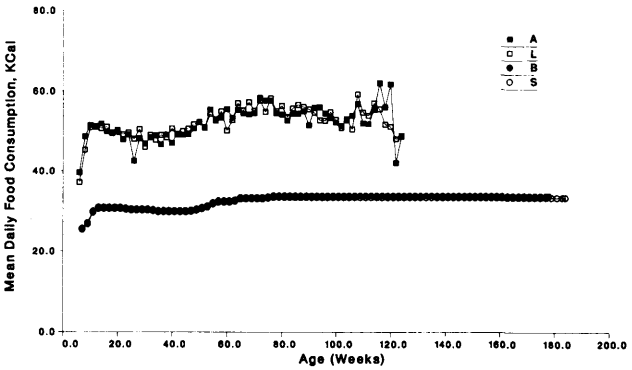

Figure 1. Food intake by rats in Groups A, L, B, and S.

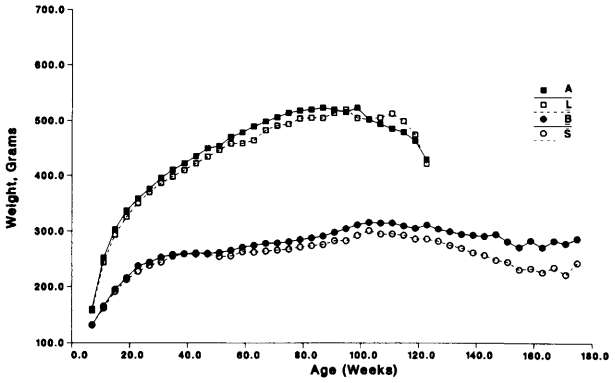

Figure 2. Body weights of rats in Groups A, L, B, and S.

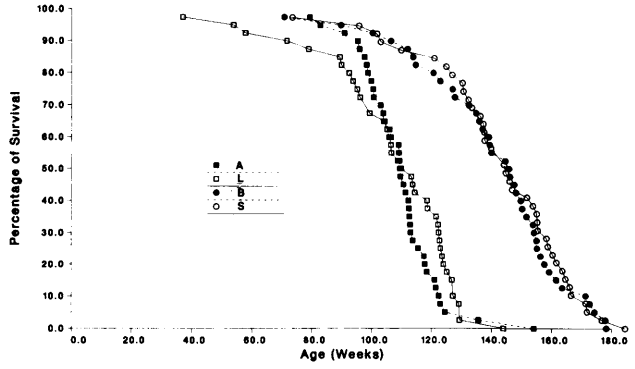

Figure 3. Survival curves for Groups A, L, B, and S.

diets with those eating ad libitum ($p < .001$), there was not a significant difference between the rats in Group A compared to Group L nor between the rats in Group B compared to Group S.

Cardiomyopathy. — Data on cardiomyopathy are reported in Table 3. Less than 10% of the rats in each group had no lesions (Grade 0) or severe lesions (Grade 3) at the time of spontaneous death. No significant difference in severity of these lesions was found between dietary groups.

Neoplastic disease. — A neoplasm that commonly occurs and plays a role in the death of some rats is pituitary adenoma. Fewer rats in Groups B and S have these tumors at the time of spontaneous death than rats in Groups A and L (Table 4; $p = .001$).

Another common neoplasm in this strain of rat which contributes to many deaths is leukemia/lymphoma. The prevalence of leukemia tended to be slightly higher in the restricted groups than in the ad libitum fed groups ($p = .067$) (Table 5).

Data on lesions in the thyroid gland, adrenal medulla, and pancreatic islets are reported in Table 6. The dietary groups did not differ markedly in the prevalence of these lesions. There tended to be a greater prevalence of pheochromocytoma at the time of death in Group B and Group S rats compared to those in Groups A and L ($p = .052$). Group A had a higher prevalence of pancreatic islet adenoma ($p = .029$).

Data on other neoplasia are presented in Table 7. The small number of most of these tumors makes it difficult to assess the influence of diet on their occurrence. However, the rats in Group A tended to have a lower prevalence of heart tumors than rats in the other three groups ($p = .067$). The number of rats with interstitial cell tumors of the testes is

Table 1. Summary of Longevity Findings

Diet Group	*n*	Median Length of Life[a]	Age of 10th Percentile Survivors[a]	Maximum Length of Life[a]
A	40	110 (104–113)[b]	123 (118–149)	154
L	40	110 (103–122)	127 (124–140)	144
B	40	146 (136–152)	171 (158–178)	178
S	39	145 (137–156)	167 (163–183)	185

[a]Age in weeks.
[b]Entries in parentheses are 95% confidence intervals.

Table 2. Severity of Chronic Nephropathy at Time of Spontaneous Death

Diet Group	*n*	Number of Rats With Lesions of Grades:[a] 0	1	2	3	4	E
A	40	0	0	8	8	8	16
L	40	1	3	6	10	11	9
B	40	10	23	5	0	1	1
S	39	18	20	0	1	0	0

[a]Grade 0 refers to no lesions; Grades 1, 2, 3, 4, and E refer to lesions in order of increasing severity.

Table 3. Severity of Cardiomyopathy at Time of Spontaneous Death

Diet Group	*n*	Number of Rats With Lesions of Grades:[a] 0	1	2	3
A	40	0	8	29	3
L	40	1	11	25	3
B	40	0	11	26	3
S	39	2	15	20	2

[a]Grade 0 refers to no lesions; Grades 1, 2, and 3 refer to lesions in order of increasing severity.

large, a well-known characteristic of this rat strain; however, rats fed the casein-containing diets (Groups A and B) appeared to have a greater prevalence ($p = .001$) of these tumors than the rats on the lactalbumin-containing diet (Group L) or the soy protein-containing diet (Group S). The restricted groups (B and S) tended to have a greater prevalence of mesothelioma than the ad libitum fed groups (A and L) ($p = .059$).

Table 4. Prevalence of Pituitary Hyperplasia and Neoplasia and Severity of Pituitary Tumors at Time of Spontaneous Death

Diet Group	n	Number of Rats With Lesions of Grades:[a] 0	H[b]	1	2	3	4
A	39	6	11	13	2	4	3
L	38	8	7	11	2	5	5
B	38	15	10	10	2	0	1
S	38	17	8	9	1	3	0

[a]Grade 0 refers to no lesions and Grade H to hyperplasia; Grades 1, 2, 3, and 4 refer to tumors in order of increasing severity.

[b]Rats having only hyperplasia.

Table 5. Prevalence and Severity of Leukemia/Lymphoma at Time of Spontaneous Death

Diet Group	n	Number of Rats With Lesions of Grades:[a] 0	1	2	3	4
A	40	26	3	2	1	8
L	40	25	5	1	0	9
B	40	19	5	4	6	6
S	39	20	5	3	3	8

[a]Lesions graded for increasing severity from Grade 0 (no lesion) to Grade 4 (most severe lesion).

Other non-neoplastic lesions. — Data on non-neoplastic lesions that appear to be influenced by diet are reported in Table 8. Rats in Groups B and S had a lower prevalence than rats in Groups A and L at the time of death of the following: cardiac mineralization ($p < .001$); esophageal hyperkeratosis ($p < .001$); gastric hyperkeratosis ($p = .032$); gastric inflammation and ulcers ($p = .004$); osteodystrophy ($p < .001$); skeletal muscle mineralization ($p < .001$); and skeletal muscle degeneration ($p = .009$). Rats in Groups A and L had a lower prevalence at the time of death of cardiac thrombosis ($p = .033$) and skeletal muscle atrophy ($p = .004$) than rats in Groups B and S.

There was little difference between the dietary groups at the time of death in the following: cardiac inflammation; blood vessel thrombus; blood vessel mineralization; kidney inflammation; kidney mineralization; hepatic inflammation; hepatic fibrosis; and impaction of the intestine (data not shown). The prevalence and severity at the time of death of hepatic bile duct hyperplasia and hepatic fatty change were not influenced by the dietary regimens studied (data not shown).

Probable causes of death. — Data on the probable causes of death are presented in Table 9. Two of these findings merit comment. First, nephropathy is a major contributor to the death of rats in Groups A and L but not of those in Groups B and S. Second, morphologic lesions indicating the cause of death were not found in 25% or more of the rats in Groups B and S; such was the case for less than 8% of the rats in Groups A and L.

DISCUSSION

Casein is not a uniquely nephropathogenic dietary protein for the male F344 rat, because the rats fed ad libitum the lactalbumin-containing diet had kidney lesions of similar severity and a similar longevity as rats fed the casein-containing diet. It may be soy protein that is unique in regard

Table 6. Prevalence of Hyperplasia and Neoplasia in Thyroid, Adrenal Medulla, and Pancreatic Islets

Diet Group	n	Thyroid gland Hyperplasia	Thyroid gland Adenoma	Adrenal Medulla Hyperplasia	Adrenal Medulla Pheochromocytoma	Pancreatic Islets Hyperplasia	Pancreatic Islets Adenoma
A	40	7	3	5	2	2	9
L	40	6	2	2	4	2	3
B	40	10	3	3	7	1	3
S	39	5	2	5	7	0	5

Table 7. Prevalence of Other Tumors at the Time of Spontaneous Death

Diet	n	Heart Tumors	Kidney Tumors	Liver Tumors	Intestinal Tumors	Mammary Gland Fibroma and Fibroadenoma	Auditory Sebaceous Gland Tumors	Interstitial Cell Tumors of Testes	Mesothelioma
A	40	0	0	0	0	6	0	31	0
L	40	3	1	0	1	2	1	20	0
B	40	2	2	1	1	3	1	33	1
S	39	5	0	0	1	0	1	24	3

Table 8. Prevalence of Other Non-neoplastic Lesions at the Time of Spontaneous Death

Diet	*n*	Cardiac Thrombus	Cardiac Mineralization	Esophageal Hyperkeratosis	Gastric Hyperkeratosis	Gastric Inflammation and Ulcers	Osteodystrophy	Skeletal Muscle Mineralization	Skeletal Muscle Degeneration	Skeletal Muscle Atrophy
A	40	1	19	33	11	17	19	18	19	6
L	40	3	16	32	15	21	11	15	16	8
B	40	7	2	17	6	8	4	5	12	18
S	39	5	2	13	8	12	1	1	7	12

Table 9. Probable Causes of Death

Disease	Dietary Group A (*n* = 40)	L (*n* = 40)	B (*n* = 40)	S (*n* = 39)
Non-neoplastic	21	15	16	16
Nephropathy	18	11	2	0
Cardiomyopathy	13	10	5	2
Impacted intestine	0	0	0	2
Inflammation	1	1	0	0
Thrombus	0	0	1	0
Undetermined	2	3	10	12
Neoplastic	16	20	23	22
Leukemia	8	9	11	11
Pituitary adenoma	6	4	1	3
Subcutaneous tumor	0	2	1	1
Cutaneous tumor	2	0	0	1
Auditory sebaceous gland tumor	0	1	1	1
Alimentary tract tumor	0	1	1	1
Endocrine tumor (other than pituitary adenoma)	0	2	3	0
Bone tumor	0	0	0	2
Mesothelioma	0	0	2	1
Other	0	1	3	1
Both neoplastic and non-neoplastic	3	5	1	1
Nephropathy	3	5	0	0
Cardiomyopathy	1	5	1	1
Leukemia	1	0	1	1
Pituitary adenoma	1	5	0	0
Subcutaneous tumor	1	1	0	0
Skin tumor	0	1	0	0
Endocrine tumor (other than pituitary adenoma)	1	0	0	0

Note. Specific diseases add up to a greater number than animals in the non-neoplastic, neoplastic, or both categories because in many animals more than one disease was felt to contribute to the death of the rat.

to the development of these kidney lesions. However, further exploration of this question cannot be readily accomplished because there are no other purified proteins available at a cost that would permit a life span dietary study.

A related question is the basis for this difference in nephropathogenesis between soy protein and the two animal proteins. Does it relate to amino acid composition or to a non-animo acid component of these purified proteins? In regard to the former, the three proteins studied have quite different amino acid compositions. Nevertheless, a particular nephropathogenic amino acid or combination of amino acids may be abundant in casein and lactalbumin but not in soy protein. Or the presence or absence of a nephropathogenic or antinephropathogenic non-amino acid substance in the animal or soy proteins may be the factor responsible. Addressing these questions experimentally would be a formidable undertaking, one outside the scope of a laboratory with a primary focus on the use of animal models in aging research.

In regard to our focus, this study shows that dietary restriction studies with male F344 rats can be effectively executed with a diet containing soy protein as its protein source. Whenever executing a dietary restriction study, it is essential to guard against the occurrence of malnutrition (Weindruch and Walford, 1988). Our findings on longevity and disease characteristics provide strong evidence that the rats in Group S did not suffer from malnutrition.

The bottom line is that the findings of the present study coupled with those of Iwasaki et al. (1988) show that the male F344 rat is an excellent model for aging studies when soy protein is the source of dietary protein. Under this condition, no single disease process is a dominant factor in the death of the rats. Iwasaki et al. showed that rats fed the soy protein diet ad libitum had many different serious lesions at the time of spontaneous death; nephropathy, cardiomyopathy, lymphoma/leukemia, and pituitary adenoma were the most common with none predominating. In the case of the Group S rats in the present study, approximately one-third of the deaths were related to leukemia/lymphoma; one-third could not be related to morphologic lesions; and the remaining third related to a spectrum of different lesions. It should also be noted that most deaths involving leukemia/lymphoma occurred at advanced ages, greater than 30 months of age. There was a high prevalence of testicular interstitial tumors in all dietary groups in this study. However, no documented death due solely to interstitial cell tumors occurred. This is not remarkable, as almost all of these tumors are benign and confined to the testicle in F344 rats (Boorman et al., 1990). Indeed, on the rare occasion of metastasis, the epididymis is the usual site of invasion.

This study further emphasizes the marked effect of restricting energy intake on age-associated diseases. In the case of nephropathy, Iwasaki et al. (1988) found that in ad libitum fed rats, 27% of those fed the soy protein diet had Grade 4 or E nephropathy at the time of death compared to 65% of the rats fed the casein diet; however, these results must be compared to the rats in Groups B and S of this study

in which Grade 4 or E lesions were present in 5% of the Group B rats and 0% of the Group S rats. Indeed, the magnitude of this finding is even more striking when it is recognized that the rats fed energy-restricted diets die at much older ages than ad libitum fed rats. Also, 42% of the rats in Groups B and S did not have pituitary hyperplasia or adenoma at the time of death compared to 18% of the rats in Groups A and L. Leukemia/lymphoma appears to be an exception, for at the time of death the prevalence of lesions was slightly higher in dietary restricted groups than in ad libitum fed groups. However, this result does not take into consideration the fact that the rats on restricted energy intake live much longer. Indeed, analyzing the data from another study in which ad libitum fed and energy-restricted male F344 rats were compared, Masoro et al. (1991) concluded that energy restriction delays the age of occurrence of leukemia/lymphoma. No significant difference was found between the dietary groups in regard to the prevalence or severity of cardiomyopathy at the time of spontaneous death. This finding also probably relates to the greater age at death of the rats on energy-restricted diets. [In a cross-sectional study of male F344 rats sacrificed at different ages, Maeda et al. (1985) showed that the progression in severity of this lesion was slowed by restricting energy intake.] Indeed, most striking is the finding that at the time of spontaneous death, 22 of the 79 Groups B and S rats showed an absence of any morphologic lesions likely to play a causal role in the death of the rat, while such was the case in only 5 of the 80 rats in Groups A and L. Moreover, in the rats of Groups B and S, 16 of the 22 deaths without significant morphologic lesions occurred at advanced ages (> 30 months) while in the rats of Groups A and L, three of the five occurred at young ages (< 24 months).

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