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The Influence of Dietary Protein Source on Longevity and Age-Related Disease Processes of Fischer Rats

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The influence of replacing dietary casein with soy protein on longevity and age-related pathologic lesions of male Fischer 344 rats was investigated. Caloric intake and body weights were similar for rats on the two diets. Rats on the soy protein-containing diet had a median length of life of 844 days compared to 730 days for those on the casein-containing diet ($p < .002$), and the ages of the 10th percentile survivors were 937 and 857 days, respectively ($p < .02$). The progression of chronic nephropathy was markedly retarded by replacing casein with soy protein. Only 7% of the rats dying spontaneously on the soy protein-containing diet exhibited end-stage chronic nephropathy compared to 41% of the rats on the casein-containing diet. Clearly, the soy protein-containing diet enables* ad libitum *fed male Fischer 344 rats to be used as a model for aging research without the occurrence of renal failure as a major confounding problem.

REDUCING the food intake of male Fischer 344 rats to 60% of that of *ad libitum* fed rats has been found to increase substantially life expectancy and life span (Yu et al., 1982; Yu et al., 1985), to slow most age-related physiologic change (Masoro, 1985; Yu et al., 1985), and to retard markedly the progression, or delay the occurrence, of the major age-related disease processes of this strain (Maeda et al., 1985). These rats were fed a standard semisynthetic diet in which casein served as the protein source, corn oil as the fat source, dextrin and sucrose as the carbohydrate source. The diet of the food-restricted rats was supplemented so that all rats had similar intakes of vitamins.

It has been suggested that protein restriction might be responsible, at least in part, for these actions of food restriction (Goodrick, 1978; Leto et al., 1976). This suggestion was tested by reducing the protein content of the diet by 40% by partially replacing the casein with dextrin (Maeda et al., 1985; Yu et al., 1985). The rats fed this diet had the same caloric intake but only 60% of the casein intake of the rats fed the standard diet. This restriction of protein caused about a 15% increase in longevity, which is small compared to the approximately 50% increase observed with food restriction involving both protein and caloric restriction (Yu et al., 1985). Moreover, age-related physiologic changes were not retarded (Kalu et al., 1983; Kalu et al., 1984; Masoro, 1984; Masoro et al., 1983). Although protein restriction in the absence of caloric restriction did not delay the occurrence of neoplastic disease, it did significantly retard the development of chronic nephropathy and cardiomyopathy but less effectively than food restriction (Maeda et al., 1985). Indeed, the small increase in longevity observed with this group of rats probably was the result of the retardation of chronic nephropathy.

A major problem interfering with the use of the male Fischer 344 rat as an animal model for aging research is renal failure due to severe chronic nephropathy, which in some rats occurs by 18 months of age and in most rats after 24 months of age (Coleman et al., 1977; Maeda et al., 1985). This problem was partially circumvented by restricting the protein intake, but such an approach suffers from a potentially marginal protein nutriture. Another possible approach is the use of a different protein source. Soy protein was chosen to be investigated in this regard, and the findings are the subject of this article.

Materials and Methods

Rat maintenance and dietary procedures. — The male Fischer 344 rats used in this study were purchased as weanlings (26 to 30 days of age) from the Kingston, NY plant of the Charles River Laboratories. To maintain the specific pathogen-free conditions, the rats, on arrival, were transferred immediately into a barrier facility where they were housed singly in plastic cages with wire mesh floors suspended on the Hazleton-Enviro Rack System (Hazleton Systems, Inc., Aberdeen, MD). The basic operations of the barrier facilities were those described previously (Yu et al., 1985). A 12:12 hr light/dark cycle was used.

Upon receipt, sentinel rats were sacrificed for the monitoring of virus antibodies (Sendai, Reo-3, GD-VII, PVM, KRV, H-1, SDA, LCM, and Adeno) and for Mycoplasma antibodies in serum sent to Microbiological Associates (Bethesda, MD). This monitoring of sentinel rats was repeated every 6 months and, in addition, rats were killed monthly for the purpose of Mycoplasma culture, Mycoplasma ELISA, RCA/SDA ELISA, and Sendai ELISA assessment by the Department of Laboratory Animal Resources of this institution. Except for the infrequent appearance of a weak positive reaction for RCV/SDA, all tests were negative.

Until 6 weeks of age, all rats were fed a semisynthetic diet (Diet A) *ad libitum;* this diet, which has been the standard

Table 1. Composition of the Diets

Components (g/100g diet)	Diet A[a]	Diet C[a]
Casein (vitamin-free)	21	0
Soy protein[b]	0	21
D,L Methionine[c]	0.15	0.35
Dextrin	43.65	43.45
Sucrose	15	15
Corn oil	10	10
Ralston-Purina vitamin mix	2	2
Choline chloride	0.2	0.2
Ralston-Purina mineral mix	5	0
Adjusted Ralston-Purina mineral mix[d]	0	5
Solka-Floc	3	3

[a]The physiologic caloric value is 4.1 Kcal/g.

[b]The soy protein in Diet C was prepared by the Ralston-Purina Company; it is purified, high quality, isolate free of fat and of trypsin inhibitor.

[c]Because both casein and soy protein have low methionine contents, the diets were supplemented with this amino acid to provide an adequate amount in both diets. To accommodate the greater methionine addition needed for Diet C, the dextrin content of Diet C was reduced to slightly below that of Diet A.

[d]The Adjusted Ralston-Purina mineral mix refers to a preparation that was modified so that Diet C had the same mineral content (including phosphorus content) as Diet A.

diet of our laboratory for more than 10 years, has casein as its protein source. (See Table 1 for composition of the diet.) At 6 weeks of age, a group of 172 rats (Group A) continued to be fed Diet A *ad libitum* for the rest of their lives; of these, 60 were designated for the longevity study and 90 for cross-sectional studies. Another group of 112 rats (Group C) was fed Diet C *ad libitum* starting at 6 weeks of age; of these, 60 were designated for the longevity study and 30 for cross-sectional studies. Diet C has soy protein as its protein source (see Table 1 for the composition of the diet).

The amount of food ingested by each rat was measured twice a week for a 3-day and 4-day period, respectively. The amount ingested per day was calculated. In order to detect the spillage of food readily, antibiotic-treated cage boards (Shepherd Specialty Papers Company, Kalamazoo, MI) were placed under the wire mesh cage floor in place of usual bedding materials. The body weight of each rat was measured at 2-week intervals.

Procedure for study of sacrificed rats. — Rats at 6, 12, 18, 24, and 27 months of age were weighed, anesthetized with ether, and sacrificed by exsanguination. The rats were examined for gross pathological lesions. The brain, pituitary gland, heart, lungs, trachea, esophagus, stomach, small intestine, colon, liver, pancreas, spleen, kidneys, urinary bladder, testes, epididymis, prostate, seminal vesicle, thyroid gland, adrenal gland, psoas muscle, sternum, and ventral abdominal skin were excised. The heart, lungs, kidneys, testes, liver, and spleen were weighed and fixed immediately in 10% neutral buffered formalin. Other tissues and organs were not weighed but were fixed immediately. Any other tissue or organ in which lesions were observed by gross inspection was excised and fixed. The fixed tissues were embedded in paraffin, sectioned at 4 μm, occasionally at 2 μm, and stained with hematoxylin-eosin with additional staining with periodic acid-Schiff's as required.

Procedures for the study of rats dying spontaneously. — All rats in the barrier facilities were inspected at least twice daily (at the start and end of the light phase of the light/dark cycle). All rats that died spontaneously were removed from their cage and either necropsied immediately or refrigerated for a brief period before necropsy. Although autolysis occurred in some cases, it did not prevent the grading of lesions.

The 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 muscle, sternum, femur, and vertebrae were excised and fixed in 10% formalin. The tissues were examined histologically by the methods just described. Any other organ or tissue with gross lesions also was examined histologically.

Grading of lesions. — Grading criteria for chronic nephropathy have been described previously (Yu et al., 1982). The following is a brief summary: Grade 0, no lesions; Grade 1, minimal severity lesions primarily involving glomerular capillary basement membrane and mesangial matrix including an occasional hyaline cast; Grade 2, lesions of mild severity involving glomerular capillary basement membrane, mesangial matrix, invariably tubular proteinaceous casts; Grade 3, moderate severity lesions involving the same structures as Grade 2 plus the thickening of Bowman's capsule, lymphocyte infiltration, and mild interstitial fibrosis; Grade 4, very severe lesions similar to those of Grade 3 but more marked, plus segmental or diffuse glomerular sclerosis and frequent adhesion of glomerular tuft to Bowman's capsule; Grade E, end-stage lesions involving widespread glomerular sclerosis, diffuse fibrosis, frequent calcification, and marked tubular dilation with numerous proteinaceous casts. Photographs of kidney sections with each grade of lesion were published in Maeda et al. (1985).

Cardiomyopathy was graded as follows: Grade 0, no lesions; Grade 1, occasional focal myocardial degeneration plus minimal fibrosis; Grade 2, frequent myocardial degeneration with extensive fibrosis; Grade 3, widespread and confluent myocardial degeneration with massive fibrosis and occasional calcification. A photograph of a heart section with Grade 3 cardiomyopathy was published in Maeda et al. (1985).

Hepatic bile duct hyperplasia was graded as follows: Grade 0, no lesions; Grade 1, increased number of bile ducts in a few portal areas (less than 10%); Grade 2, increased number of bile ducts in 10 to 30% of portal areas plus occasional fibrosis and lymphocytic infiltration; Grade 3, increased number of bile ducts in more than 30% of portal areas plus extensive fibrosis. A photograph of a liver section with Grade 3 bile duct hyperplasia was published in Maeda et al. (1985).

Hepatic fatty change was graded as follows: Grade 0, no lesions; Grade 1, a few small fat droplets in hepatocytes near portal area; Grade 2, many moderate-sized fat droplets in

hepatocytes near portal area and in mid-zonal region; Grade 3, many large fat droplets in hepatocytes throughout liver. A photograph of a liver section with Grade 3 lesion was published in Maeda et al. (1985).

Lesions were routinely graded microscopically by one of the pathologists without reference to the code number of the rat. A second pathologist reviewed the slides from randomly selected animals. In the case of the chronic nephropathy, a test of the reliability of the grading was made by submitting to both pathologists a collection of slides from 20 rats representing different grades of lesions. The slides were identified by a code that was not known to the pathologists. They were examined, and the slides were scored independently. The two pathologists gave the same grade to slides from 17 of the 20 rats, and in the case of the other 3 rats there was a 1-grade disagreement in the 6-grade system.

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

When disease state was assessed using multiple-ordered categories, the progression of disease in sacrificed animals was analyzed using ridit analysis (Fleiss, 1973), with the reference group being the marginal distribution for the rats fed Diet A *ad libitum* reported by Maeda et al. (1985). When the disease state was a binary variable, the disease progression was analyzed using a chi-square analysis for trends in proportions (Fleiss, 1973). For the animals that died spontaneously, the total frequency of disease states was analyzed using a chi-square test (Siegel, 1956). When the expected frequencies were too small for the chi-square test, the data were analyzed using Fisher's exact test (Siegel, 1956) for 2 × 2 tables.

RESULTS

Food intake. — The intake of food expressed as Kcal per rat per day is presented in Figure 1. There was no significant difference in caloric intake between the two groups; neither did the intake change appreciably with age.

Body and organ weights. — The body weights of the Group A and Group C rats are presented in Figure 2. Both groups had similar body weights through 90 weeks of age. After 90 weeks of age, Group A rats began to lose body weight; such a loss did not occur in the Group C rats until after 120 weeks of age.

The organ weights of the Group A rats sacrificed at 6, 12, 18, and 24 months of age were similar to those reported in an earlier study (Yu et al., 1985) for male Fischer 344 rats fed this diet. The organ weights of the Group C rats were similar to those of the Group A rats for all ages studied in common.

Longevity. — The survival curves for the Groups A and C

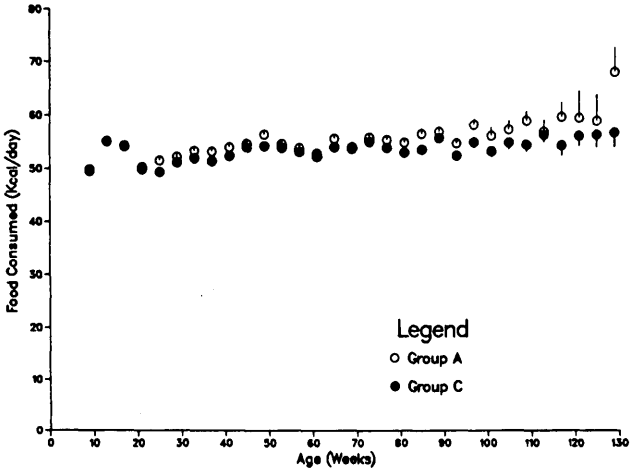

Figure 1. Food intake (Kcal per rat per day) by Group A and Group C rats of the longevity study. The points are means (bars represent standard errors of the means); the number of rats at the start of the study was 60 for each group and decreased with age as indicated by the survival curves in Figure 3.

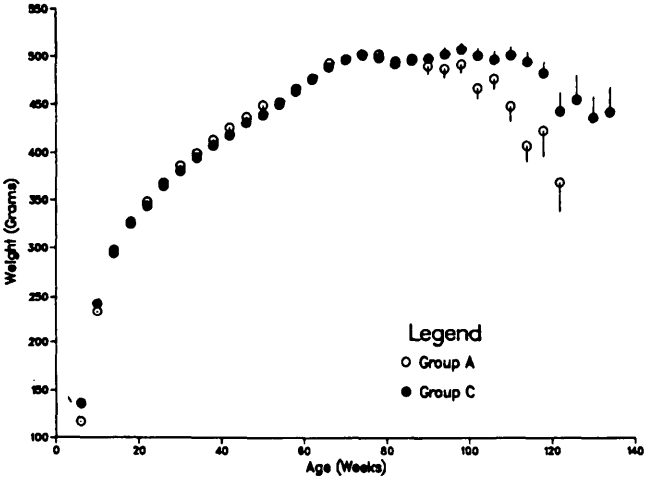

Figure 2. Body weights of Group A and Group C rats of the longevity study. The points are means (bars represent standard errors of the means); the number of rats at the start of the study was 60 for each group and decreased with age as indicated by the survival curves in Figure 3.

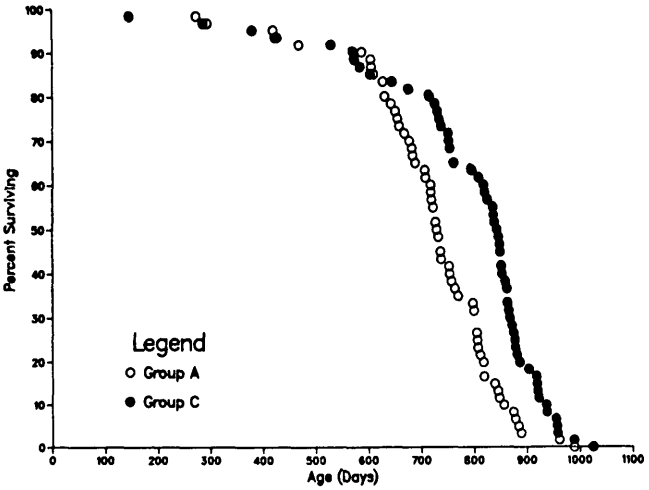

Figure 3. Survival curves for Group A and Group C rats. Both groups had 60 rats at the start of the study.

rats are shown in Figure 3. The curve for the Group C rats differs significantly from that of the Group A rats (p = .0002). The median length of life and the age of the 10th percentile survivors are presented in Table 2. The median length of life was significantly greater for Group C rats than for the Group A rats ($p < .002$). The age of the 10th percentile survivors also was significantly greater for Group C rats than for Group A rats ($p < .02$).

Chronic nephropathy. — Data on the severity of chronic nephropathy of rats sacrificed at 6, 12, 18, 24, and 27 months of age are reported in Table 3. The severity of these lesions increased with age in both groups (Group A, p = .027; Group C, p = .059), each showing a significant linear trend in severity with age (Group A, p = .011; Group C, p = .018). At the three ages studied in common (18, 24, and 27 months of age), the severity of lesions in Group C was less than in Group A ($p <$ .037). When the data were combined across these ages, the severity of the lesions in the rats of Group A was significantly greater than that of the rats of Group C (p = .0001). Particularly striking is the fact that 4 of the 33 rats 18 months of age or older of Group A had Grade E lesions, whereas none of the 24 rats in Group C had lesions of this severity. Data on the severity of chronic nephropathy in all rats that died spontaneously are reported in Table 4; the rats in Group A had much more severe lesions than those in Group C ($p < .0001$). Indeed, 46 of the 111 rats that died spontaneously in Group A had Grade E lesions, whereas only 5 of the 70 Group C rats had lesions of this severity at death.

Cardiomyopathy. — The data on cardiomyopathy of rats sacrificed at various ages (Table 5) indicated an increase in severity of lesion with age in both groups (Group A, p = .002; Group C, p = .0009). At the three ages studied in common the severity of those lesions in Group C rats was similar to that found in Group A rats. Data on the severity of cardiomyopathy in all rats that died spontaneously are reported in Table 6; the rats in Groups A and C exhibited similar severity of lesions.

Gastrointestinal lesions. — The Group A rats had the type and extent of gastrointestinal lesions (Table 6) reported previously for male Fischer 344 rats fed this diet (Maeda et al., 1985). The rats in Group C exhibited similar lesions but had a lower frequency of gastric hyperkeratosis (p = .014) and gastric ulcers (p = .044).

Hepatic lesions. — The Group A rats that died spontaneously had the type and extent of hepatic lesions (Table 6) reported previously for male Fischer 344 rats fed this diet (Maeda et al., 1985). The rats in Group C were similar to Group A in regard to hepatic lesions.

Reproductive lesions. — The Group A rats that were sacrificed and those that died spontaneously had the type and extent of reproductive system lesions (Tables 5 and 6) reported previously for male Fischer 344 rats fed this diet (Maeda et al., 1985). The rats in Group C were similar to Group A in regard to reproductive system lesions.

Table 2. Summary of Longevity Findings[a]

Findings	Diet groups	
	Diet A	Diet C
Median length of life	730(708-764)	844(809-863)
Age of 10th percentile	857(819-961)	937(918-989)
Maximum length of life	989	1,025

Note. Entries are days; 95% confidence intervals are in parentheses. n = 60 in both diet groups.

[a]Upon arrival of the weanling rats (4 weeks of age), 150 were randomly selected for Group A, 90 for Group C. From each group, 60 rats were randomly selected for the longevity study, and the rest were used for cross-sectional studies. All rats that died spontaneously (longevity plus cross-sectional) were analyzed for pathologic lesions; however, only the 60 rats of each group designated for the longevity analysis were used in developing the survival curves for the data presented in this table.

Table 3. Severity of Chronic Nephropathy in Rats Sacrificed at Various Ages, by Diet Group

Ages (in months)	Number examined	Number with lesions of grades					
		0	1	2	3	4	E
Diet Group A							
6	18	3	15	0	0	0	0
12	10	0	2	6	2	0	0
18	15	0	0	2	10	1	2
24	10	0	0	1	5	4	0
27	8	0	0	0	2	4	2
Diet Group C							
18	7	0	0	6	1	0	0
24	10	0	0	3	7	0	0
27	7	0	0	2	3	2	0

Note. See text for descriptions of lesion grades.

Table 4. Severity of Chronic Nephropathy in Rats Dying Spontaneously by Age and Diet Group

Ages (in months)	Number examined	Number with lesions of grades					
		0	1	2	3	4	E
Diet Group A							
< 12	4	0	3	1	0	0	0
12 to 18	9	0	3	6	0	0	0
18 to 24	60	0	0	6	15	14	25
24 to 30	36	0	0	1	4	11	20
> 30	2	0	0	0	0	1	1
Total	111	0	6	14	19	26	46
Diet Group C							
< 12	2	2	0	0	0	0	0
12 to 18	5	0	5	0	0	0	0
18 to 24	13	0	1	7	4	0	1
24 to 30	39	0	2	9	16	10	2
> 30	11	0	0	1	4	4	2
Total	70	2	8	17	24	14	5

Note. See text for descriptions of lesion grades.

Table 5. Lesions Other Than Chronic Nephropathy in Rats Sacrificed at Various Ages by Diet Group

Types of Lesions	6 months of age	12 months of age	18 months of age		24 months of age		27 months of age	
	Group A (n = 18)	Group A (n = 10)	Group A (n = 15)	Group C (n = 7)	Group A (n = 10)	Group C (n = 10)	Group A (n = 8)	Group C (n = 7)
Cardiomyopathy								
Number with Grade 0 lesions	11	3	3	2	0	0	0	0
Number with Grade 1 lesions	7	7	10	5	6	6	2	3
Number with Grade 2 lesions	0	0	2	0	3	3	4	3
Number with Grade 3 lesions	0	0	0	0	1	1	2	1
Number with reproductive system lesions								
Testicular interstitial cell								
Hyperplasia	0	0	9	7	8	5	6	5
Tumors	0	0	11	3	9	8	8	7
Atrophy of seminiferous tubules	1	1	3	2	9	8	8	7
Testicular calcium deposits	0	2	2	2	3	6	6	3
Acute or Chronic prostatitis	0	0	1	0	0	0	3	3
Number of tumor-bearing rats[a]								
Benign	0	0	8	2	7	4	5	3
Malignant	0	0	0	2	1	1	3	2
Total	0	0	8	3	7	5	6	4
Number with epithelial tumors[b]								
Benign	0	0	2	0	0	0	2	1
Malignant	0	0	0	0	0	0	0	1
Number with mesenchymal tumors[b]								
Benign	0	0	1	1	0	1	0	0
Malignant	0	0	0	0	0	0	0	0
Number with selected nonendocrine specific tumors								
Preputial gland	0	0	1	0	3	0	0	0
Lymphoma or leukemia	0	0	0	0	0	1	2	0
Mesothelioma	0	0	0	0	0	0	0	0
Number with endocrine tumors								
Pituitary adenoma	0	0	3	0	3	2	4	2
Thyroid C-cell tumors	0	0	0	3	2	0	2	3
Thyroid follicular tumors	0	0	0	0	0	0	1	1
Adrenal cortical tumors	0	0	1	0	0	0	1	0
Pheochromocytoma	0	0	0	0	0	2	0	0
Pancreas Islet cell tumors	0	0	1	1	4	2	1	0

[a]Tumors other than testicular interstitial cell tumors.
[b]Other than those listed under the headings of selected nonendocrine-specific tumors or endocrine gland tumors.

Neoplastic lesions. — Neoplastic lesions in the Group A and Group C rats that were sacrificed and that died spontaneously are reported in Tables 5 and 6. As was the case in a previous study (Maeda et al., 1985), neoplastic lesions were not observed during the first year of life. There were few differences between Group A and Group C in the occurrence of neoplastic lesions. A greater percentage of Group C rats than Group A rats that died spontaneously had benign (p = .0005) and malignant mesenchymal tumors (p = .005) and lymphomas or leukemia (p = .01). The occurrence of tumors of the endocrine glands was similar for Group A and Group C rats except that a higher percentage of Group C rats that died spontaneously had pancreatic islet cell tumors (p = .05).

Miscellaneous nonneoplastic lesions. — Data on the following lesions in rats that died spontaneously are reported in Table 6: osteodystrophy; calcium deposition in heart, kidney, and muscle; pancreatic lobular atrophy; and thyroid C-cell hyperplasia. A significantly smaller percentage of Group C rats exhibited osteodystrophy (p = .0004), thyroid C-cell hyperplasia (p = .02), and calcium deposits in heart (p = .0008), kidney (p = .02), and muscle (p = .0006) than was the case for Group A rats. As discussed previously (Maeda et al., 1985), the osteodystrophy is probably secondary to the renal disease. (See that article for an in-depth description.)

DISCUSSION

The most striking finding of this study is the marked retardation in the development of chronic nephropathy in male Fischer 344 rats fed the soy protein diet *ad libitum*. Chronic nephropathy, involving glomerular sclerosis, mesangial cell injury, and mesangial matrix over production, is commonly found in most strains of *ad libitum* fed rats at advanced ages (Anver and Cohen, 1979). Similar progressive lesions also are observed in young rats following surgical reduction of kidney mass (Chanuten and Ferris, 1932; Purkerson et al., 1976; Shimamura and Morrison, 1975) and in humans following disease-induced kidney damage after

Table 6. Lesions Other Than Chronic Nephropathy in Rats Dying Spontaneously at Various Ages by Diet Group

	< 12 months of age		12 to 18 months of age		18 to 24 months of age		24 to 30 months of age		> 30 months of age		Total	
Types of Lesions	Group A (n = 4)	Group C (n = 2)	Group A (n = 9)	Group C (n = 5)	Group A (n = 60)	Group C (n = 13)	Group A (n = 36)	Group C (n = 39)	Group A (n = 2)	Group C (n = 11)	Group A (n = 111)	Group C (n = 70)
Cardiomyopathy												
Number with Grade 0 lesions	2	0	2	2	3	1	0	2	0	0	7	5
Number with Grade 1 lesions	2	2	7	3	23	8	8	18	0	2	40	33
Number with Grade 2 lesions	0	0	0	0	25	4	18	13	1	7	44	24
Number with Grade 3 lesions	0	0	0	0	9	0	10	6	1	2	20	8
Number with lesions of gastrointestinal tract												
Esophageal hyperkeratosis	0	0	5	2	31	12	24	23	2	6	62	43
Gastric hyperkeratosis	1	0	0	1	28	2	22	14	1	3	52	20
Gastric ulcer	0	0	0	0	21	2	19	12	1	2	41	16
Intestinal obstruction by hair balls	3	0	6	4	2	1	0	1	0	1	11	7
Number with hepatic lesions												
Bile duct hyperplasia												
Number with Grade 0 lesions	4	2	3	0	0	0	0	0	0	0	7	2
Number with Grade 1 lesions	0	0	5	5	28	8	14	16	0	4	47	33
Number with Grade 2 lesions	0	0	1	0	21	3	15	15	2	7	39	25
Number with Grade 3 lesions	0	0	0	0	11	2	7	8	0	0	18	10
Fatty change												
Number with Grade 0 lesions	1	1	2	1	14	6	8	6	1	3	26	11
Number with Grade 1 lesions	3	1	6	4	32	7	20	24	0	3	61	39
Number with Grade 2 lesions	0	0	1	0	8	4	5	6	0	2	14	12
Number with Grade 3 lesions	0	0	0	0	6	2	3	3	1	3	10	8
Periductal fibrosis	0	0	0	0	38	6	31	29	1	8	70	43
Cystic space	1	0	0	0	6	0	8	7	1	1	16	8
Number with reproductive systems lesions												
Testicular interstitial cell												
Hyperplasia	0	0	1	1	28	6	14	12	0	0	43	19
Tumors	0	0	0	0	46	8	35	38	2	11	83	57
Atrophy of seminiferous tubules	0	0	1	0	35	5	32	32	2	11	70	48
Testicular calcium deposits	0	0	0	0	23	4	18	19	1	5	42	28
Acute or chronic prostatitis	3	1	1	2	14	1	10	11	2	2	30	17
Number of tumor-bearing rats[a]												
Benign	0	0	2	0	34	6	24	30	2	8	62	44
Malignant	0	0	1	1	24	8	16	25	0	7	41	41
Total	0	0	3	1	46	12	31	37	2	10	82	60
Number with epithelial tumors[b]												
Benign	0	0	0	0	4	0	3	2	1	2	8	4
Malignant	0	0	0	0	5	0	3	3	0	1	8	4
Number with mesenchymal tumors[b]												
Benign	0	0	0	0	2	1	0	8	0	2	2	11
Malignant	0	0	1	1	4	3	0	7	0	1	5	12
Number with selected non-endocrine-specific tumors												
Preputial gland	0	0	0	0	11	0	3	3	0	2	14	5
Lymphoma or leukemia	0	0	0	0	5	2	2	10	0	1	7	13
Mesothelioma	0	0	0	0	4	0	0	5	0	1	4	6
Number with endocrine tumors												
Pituitary adenoma	0	0	1	0	15	5	10	8	1	3	27	16
Thyroid C-cell tumors	0	0	1	0	8	2	8	8	0	3	17	13
Thyroid follicular tumors	0	0	0	0	2	0	2	1	0	0	4	1
Adrenal cortical tumors	0	0	0	0	3	0	1	3	0	1	4	4
Pheochromocytoma	0	0	0	0	2	0	5	7	1	2	8	9
Pancreas Islet cell tumors	0	0	0	0	5	1	6	12	1	2	12	15

(continued)

Table 6. Lesions Other Than Chronic Nephropathy in Rats Dying Spontaneously at Various Ages by Diet Group (continued)

Types of Lesions	< 12 months of age		12 to 18 months of age		18 to 24 months of age		24 to 30 months of age		> 30 months of age		Total	
	Group A (n = 4)	Group C (n = 2)	Group A (n = 9)	Group C (n = 5)	Group A (n = 60)	Group C (n = 13)	Group A (n = 36)	Group C (n = 39)	Group A (n = 2)	Group C (n = 11)	Group A (n = 111)	Group C (n = 70)
Number with selected non-neoplastic lesions												
Osteodystrophy	0	0	0	0	16	0	16	3	1	2	33	5
Calcium deposits in												
Heart	0	0	0	0	12	2	13	1	1	0	26	3
Kidneys	4	1	2	4	34	7	29	15	1	5	70	32
Muscles	0	0	0	0	21	1	20	7	2	2	43	10
Pancreatic lobular atrophy	0	0	2	2	18	3	7	10	0	4	27	19
Thyroid C-cell hyperplasia	0	0	0	0	21	2	15	8	0	2	36	12

[a]Tumors other than testicular interstitial cell tumors.
[b]Other than those listed under the headings of selected nonendocrine-specific tumors or endocrine gland tumors.

the initial disease process is no longer active (Brenner et al., 1982). In another article by researchers from our laboratory (Maeda et al., 1985), it was reported that the age-related development of chronic nephropathy in male Fischer 344 rats was retarded by reducing protein intake without changing caloric intake. The nephropathy that follows the surgical reduction of kidney mass also is slowed by decreasing dietary protein (Hostetter et al., 1981; Laonari et al., 1983; Moise and Smith, 1927). Moreover, there is evidence to indicate that reducing the protein intake of humans slows the progression of chronic renal failure to end-stage disease (Mitch, 1984). Brenner et al. (1982) suggested that decreased dietary protein acts by preventing glomerular hyperperfusion and hyperfiltration, which they believe to be responsible for the progression of these lesions and for the eventual loss of renal function.

The important point from our results is that the soy protein diet enables almost all *ad libitum* fed male Fischer 344 rats to live out their life without either the occurrence of renal failure or the potential problem of inadequate protein nutrition. In a previous study (Maeda et al., 1985), it was found that renal failure occurred in the rats with Grade E chronic nephropathy lesions but not with the other grades of lesions. In the case of the rats fed the casein diet (Group A), 41% of the rats dying spontaneously had Grade E lesions, whereas only 7% of the rats on the soy protein diet (Group C) had such lesions. Moreover, only 18% of the Group C rats dying at 30 months or older ages had Grade E lesions. The male Fischer 344 rat has been a major animal model for aging research because of its genetic homogeneity and because it does not develop the obesity that occurs with advancing age in many rat strains (Committee on Animal Models For Research on Aging, 1981). However, a major problem with this animal model has been renal failure at advanced ages, which has precluded the study of normal aging processes in some of the rats by 18 months of age and in many of the rats after 24 months of age. To a great extent, soy protein as the dietary protein source circumvents this problem, thereby facilitating full life-span studies with this animal model without the confounding problem of renal failure.

The findings in this article might also have a bearing on the treatment of patients with chronic renal failure. Although restricting the dietary protein of such patients retards the further loss of renal function and prolongs the time before dialysis or transplantation is needed, it has the risk of protein malnutrition as well as mineral and vitamin depletion (Mitch, 1984). Our findings indicate that the proper selection of protein source may yield a treatment for the chronic renal failure patient as effective as protein restriction without its risks.

The soy protein diet (Group C) compared to the casein diet (Group A) did significantly increase median length of life by 114 days (from 730 to 844 days) and that of the 10th percentile survivors by 80 days (from 857 to 937 days). In this and two separate studies (Yu et al., 1982; Yu et al., 1985), using the casein diet and carried out over the past 12 years, the median length of life in days was 711 (n = 115), 701 (n = 40), and 730 (n = 60), and the age in days of the 10th percentile survivors was 797 (n = 115), 822 (n = 40), and 857 (n = 60). Thus, the effect of the soy protein diet on longevity is small; this is underscored by the action of food restriction, which increased the median length of life by 356 days (from 701 to 1,057 days) and that of the 10th percentile survivors by 404 days (from 822 to 1,226 days) in a study in which rats eating Diet A *ad libitum* were compared with those provided that diet at 60% of the mean *ad libitum* intake (Yu et al., 1985). Of course, the food intake of the Group C rats was the same as that of the Group A rats, and, thus, no element of food restriction was involved in the present study. Nevertheless, it is striking that renal failure could be so greatly reduced without markedly influencing longevity. Clearly, food restriction must increase longevity by actions in addition to its ability to retard the development of chronic nephropathy.

The question arises as to what pathology occurs in the soy protein fed rats (Group C) that contributes to death. The data of this study show that in Group C rats there were lesions in addition to chronic nephropathy that were decreased compared to Group A rats, specifically: gastric ulcers; osteodystrophy; calcium deposition in heart, kidneys, and muscle; gastric hyperkeratosis; and thyroid C-cell hyperplasia. On the basis of our previous work (Maeda et al., 1985), the first three are probably secondary to the decrease in the progression of chronic nephropathy. However, at death the follow-

ing lesions were more prevalent in Group C rats than Group A rats: benign and malignant mesenchymal tumors (especially subcutaneous soft tissue tumors), lymphomas or leukemia, and pancreatic islet cell tumors. Because the data from the cross-sectional studies on the rats sacrificed at 18, 24, and 27 months of age did not show the Group C rats to have these tumors to a greater extent than Group A rats, it seems likely that the reason for the higher prevalence of these lesions at the times of spontaneous death relates to the fact that the Group C rats live longer. Thus, it is reasonable to hypothesize that retarding the development of chronic nephropathy does not increase longevity markedly because of the occurrence of other disease processes with advancing age. Food restriction markedly extends longevity because of its broad spectrum of action on age-related diseases and on physiologic deterioration (Masoro, 1984) in addition to its action on the progression of chronic nephropathy.

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