Journal of Gerontology: BIOLOGICAL SCIENCES
1993, Vol. 48, No. 6, B213–B230

Pathological Characterization of Male Wistar Rats From The Gerontology Research Center

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Male Wistar rats aged 6–26 months were obtained from the colony of The Gerontology Research Center of the National Institute on Aging, and pathological profiles were assessed. One hundred animals were sacrificed at 6, 12, 18, 21, 24, and 26 months and used for cross-sectional determinations; also, 150 animals were followed longitudinally and sacrificed when clinical signs of moribundity appeared. Renal disease contributed the most common pathology observed in both studies (found in over 70% of the animals examined), with neoplasms a secondary problem (pituitary tumors were by far the most prevalent, with adenomas present in ≈ 20% of the animals). This analysis represents the first complete pathological characterization of this commonly used rat model for aging research, and offers an opportunity for comparison with other rat strains.

THE most frequently employed animal models for studying basic mechanisms of aging are rodents (Rowlatt et al., 1976; Coleman et al., 1977; Goodman et al., 1979; Anver et al., 1982; Malkowa et al., 1983; Rehm et al., 1984; Maeda et al., 1985; Boorman et al., 1990; Bronson, 1990). Although it is often difficult to distinguish "normal" from "pathological" aging, gerontologic investigators are charged with determining whether manifestations of "normal" aging exhibited by such models are secondary to disease, life style, or other factors peculiar to specific species/strains. Since aging of rodents shows both parallels and contrasts with that of humans, a number of rat and mouse strains commonly used in gerontology have been pathologically characterized over the past 15 years (Rowlatt et al., 1976; Coleman et al., 1977; Goodman et al., 1979; Anver et al., 1982; Malkowa et al., 1983; Rehm et al., 1984; Maeda et al., 1985). The Fischer 344 strain rat was one of the first to have its pathology extensively described and has consequently become the most popular rat model for the broad gerontological community (Coleman et al., 1977; Goodman et al., 1979; Maeda et al., 1985). Various other rat and mouse strains have subsequently been characterized and have been utilized by specific investigators for particular purposes (Rowlatt et al., 1976; Anver et al., 1982; Malkowa et al., 1983; Rehm et al., 1984).

A frequently employed aging rat model over the last two decades has been the outbred Wistar strain maintained at the N.W. Shock Laboratories of The Gerontology Research Center, NIA, in Baltimore, Maryland. This colony has been used to support NIA intramural research as well as a number of collaborative, extramural investigations. Although a number of physiological (Guarnieri et al., 1980; Roth et al., 1981), biochemical (Ito et al., 1982), and behavioral (Joseph et al., 1978, 1983) age changes have been described in this rat model, pathological characterization has been limited to specific studies and has not been attempted comprehensively (e.g., Hirokawa, 1975). Consequently, the present study was conducted with two objectives: (1) To determine the major causes of death of animals within the colony; (2) To characterize the pathologic changes occurring in organ systems as a result of aging.

Materials and Methods

The Gerontology Research Center Aging Wistar Rat Colony originated in the summer of 1969, when 200 virgin females and 100 virgin male rats were obtained from the Wistar Institute to establish the nucleus of the breeding colony. To maintain the outbred quality of the rats, a random breeding regime was established, with 1 male and 2 females paired together until pregnancy could be determined. Prior to parturition, the gravid female was placed in a stainless steel wire-bottom cage, with a stainless steel floor plate, and shredded newspaper for bedding. Soiled paper was changed twice weekly. Litters were weaned at 21 days of age and ear-punched. The breeding female rat was returned to the male's cage.

After a few years of random matings, the breeding program began to have problems with productivity, with fewer female breeders becoming pregnant and reduced litter sizes. Because we chose not to introduce any new rats from outside the colony for breeding, Dr. Carl Hansen, the NIH staff geneticist, implemented a new breeding system. Family lines "A" through "L" were established for the existing breeding program, approximately 200 pairs. The male from the "A" family line was always mated to a "B" female, and so forth down the family line. Litters born receive the family line letter of the female parent. This system was thought to maximize the heterogeneity of the colony and is still in use today. Based on the high productivity and low mortality of our breeding program, we believe that any inbreeding coefficients have been minimized. (For reference to the circular pair mating system, see Hansen et al., 1981.)

In 1978 we began a breeding system in which one male was paired with two females in a plastic cage for the duration of their productivity. These animals are selected at weaning and identified with an ear punch, indicating the family line. Each group begins producing pups at approximately 72 days of age. As based on productivity studies, animals are allowed to breed until 6 months of age, at which time the male is removed from the group. The females are allowed to raise any remaining pup and are then retired.

To maximize the male pups' survival, female pups are culled at birth, leaving two female pups for potential breeders and colony animals. After the pups are weaned, they are ear-punched with the appropriate cohort identification, selected as breeders, or culled if of inferior quality.

This colony is sequestered in the basement of the GRC, in 13 holding rooms. The rats are housed in stainless steel suspended cages of 305 square inches floor space with hyperchlorinated water (10 ppm) and fed, ad libitum, the NIH 07 Open Formula Diet. This diet has a guaranteed analysis of 23.5% (minimum) crude protein, 4.5% (minimum) crude fat, and 4.5% (maximum) crude fiber. Room temperature was maintained at 72° ± 2°. The room humidity was 45%, and the photoperiod was 12 hr light (0600 hr) and 12 hr dark (1800 hr).

Two groups of male Wistar rats (100 rats per group) were utilized to conduct cross-sectional and longitudinal studies.

Cross-Sectional Study (C-SS)

A selected number of rats of different ages from 6 months to 27 months (six separate groups) were drawn randomly from the colony at large. There were 15 rats each in the 6-month and 12-month groups; 20 rats each in the 18-month, 21-month, and 24-month groups; and 10 rats in the 26-month group. The rats were chosen randomly without regard to clinical signs of health status.

Longitudinal Study (LS)

One hundred fifty rats approximately one year old were set aside in the GRC colony and maintained as a cohort. The rats were monitored daily for clinical signs of moribundity. These signs included loss of up to 20% in body weight, depression-like behavior, ataxia, anorexia, paralysis, and dyspnea. Rats were immediately sent to the pathology contractor when their moribund condition was established. The first 100 rats euthanized for necropsy and evaluation were included in the LS group.

All rats were put into a state of narcosis with carbon dioxide, euthanized by exsanguination from the abdominal aorta, and subjected to a complete necropsy prior to removal of all organs/tissues. Required tissues and all gross lesions were collected from each rat and fixed in 10% neutral buffered formalin. After at least 72 hours in the fixative, the tissues were trimmed, processed conventionally and embedded in paraffin, sectioned at approximately 6 μm, stained with hematoxylin and eosin (H&E), and evaluated microscopically by board-certified pathologists at Pathology Associates, Inc. (Frederick, MD).

A complete blood count was performed on each blood sample. Standard viral/mycoplasmal screens were run on approximately one-half of the samples by Microbiological Associates (Rockville, MD). These screens included hemagglutination inhibition (HI) tests for Reovirus type 3 (Reo3), Kilham rat virus (KRV), and Toolan's H-1 virus (H-1); ELISA tests for Cilia-associated respiratory bacillus (Carb), Sendai virus (Send), Pneumonia virus of mice (PVM), Rat coronavirus/sialodacryoadenitis virus (RCV/SDA), and *Mycoplasma pulmonis* (M.Pul.); and immunofluorescent antibody (IFA) test for lymphocytic choriomeningitis (LCM).

RESULTS

Clinical Pathology

Serology

Positive serologic titers were found only for RCV/SDA (52/52 and 48/48), PVM (41/52 and 46/48), H-1 (8/52 and 41/48), and KRV (8/52 and 18/48). While all rats were positive for RCV/SDA, it is noteworthy that the incidence of rats positive for PVM and KRV is somewhat lower in the cross-sectional study, and the incidence of positive H-1 rats is much lower in the C-SS. In addition, in the C-SS, all positive KRV rats and 6/8 positive H-1 rats were 18 months of age or older. The remaining two positive H-1 rats were 12 months old.

In these studies, no lesions indicative of SDA were encountered in the salivary or lacrimal glands. However, infection with SDA may have been manifested in the nose of some rats. The potential effects of PVM and RCV would, if present, be manifested in the lung. These will be discussed with the histopathologic findings in the lungs and nose. Nematode parasites (pinworms) were present in the colons and/or ceca of rats of all ages in both studies. No histologic lesions could be attributed to them.

Hematology

Hematology data of the C-SS rats are presented by group in Table 1. As is apparent, several of the hematologic values were age-related. Individual data for each variable were submitted to a linear regression onto age. The proportion of variance (R^2) explained by age is shown in Table 1 for each variable with significant regression accepted at $p < .01$. With increased age, the relative number of neutrophils increased while the relative number of lymphocytes decreased. The red blood cell count, hemoglobin, and hematocrit generally decreased with increased age.

Moribundity in the LS. — The incidence of moribundity among rats in the LS is depicted in Figure 1. From this figure, the median age of moribundity has been calculated to be approximately 19 months. Animals seldom lived more than one month after attaining a moribund condition, and median mortality in this colony has ranged historically from 20–24 months (unpublished data). To more accurately define the point at which age-related changes were initially observed, the 100 moribund-sacrificed rats were divided initially into four age groups: 13–18 months, 19–20 months, 21–22 months, and 23–24 months.

Based upon gross and microscopic lesions, a determination of the probable cause of moribundity was made (where possible) for each animal. These are itemized in Table 2. As

Table 1. Cross-sectional Study, Hematology by Age Group; Mean Values and Standard Deviations

	6-Month (14)	12-Month (15)	18-Month (19)	21-Month (20)	24-Month (20)	27-Month (8)	R^2
WBC (10^3/mm^3)	11.2 (2.0)	9.8 (2.0)	11.6 (3.0)	12.6 (0.9)	11.4 (2.5)	12.7 (3.0)	.04
Neutrophil (%)	16.1 (4.4)	22.3 (6.5)	34.8 (12.2)	39.1 (14.0)	39.3 (16.4)	39.4 (13.9)	.32*
Lymphocyte (%)	79.1 (5.2)	73.2 (7.2)	63.4 (10.5)	61.1 (11.5)	58.9 (15.3)	58.8 (14.2)	.30*
EOS (%)	1.8 (1.5)	0.9 (1.1)	0.9 (1.2)	1.0 (1.1)	1.0 (0.8)	0.4 (0.5)	.06
Monocycle (%)	1.6 (1.2)	1.5 (2.1)	1.5 (1.5)	1.6 (1.8)	1.7 (1.5)	1.5 (1.9)	.00
RBC (10^6/mm^3)	8.5 (0.5)	8.0 (0.4)	7.5 (1.2)	7.1 (0.8)	7.8 (1.0)	6.8 (0.9)	.14*
Hemoglobin (GM/DL)	14.6 (0.6)	13.8 (0.4)	13.0 (2.1)	12.4 (1.6)	13.6 (1.8)	12.1 (1.4)	.12*
Hematocrit (%)	44.5 (2.9)	41.7 (2.0)	39.0 (6.7)	37.0 (5.0)	41.2 (5.7)	37.5 (4.4)	.11*
MCV (micron^3)	52.5 (1.8)	52.6 (2.3)	52.0 (2.4)	52.3 (2.7)	52.8 (2.0)	55.0 (3.0)	.02
MCH (picogram)	17.3 (0.6)	17.4 (0.9)	17.4 (0.9)	17.5 (1.0)	17.4 (0.8)	17.7 (1.0)	.01
MCHC (%)	33.1 (0.8)	33.1 (1.1)	33.4 (0.8)	33.5 (0.8)	33.0 (1.0)	32.2 (0.4)	.00
Platelets (10^4/mm^3)	108.2 (22.1)	118.6 (9.1)	122.8 (29.2)	135.3 (24.9)	116.4 (26.7)	117.4 (25.0)	.02

Notes. WBC = white blood cells; EOS = eosinophil; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

*$p < .01$.

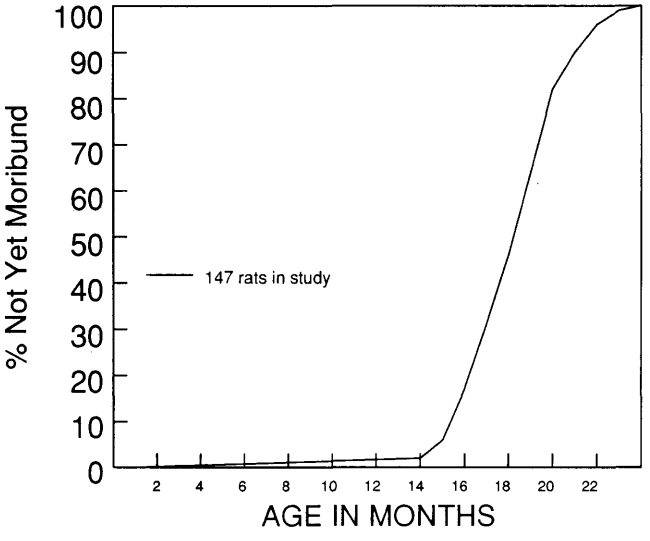

Figure 1. Longitudinal morbidity study, 1988–1990.

Table 2. Longitudinal Study, Summary Mortality Data by Age Group

Diagnoses	<13 Months $n = 0$	13–18 Months $n = 52$	19–24 Months $n = 48$	>24 Months $n = 0$
Undetermined	0	2	5	0
Renal failure	0	44	25	0
Zymbal's gland tumor	0	0	2	0
Atrial thrombosis and lung inflammation	0	1	1	0
Thyroid tumor	0	0	1	0
Renal failure and pituitary tumor	0	0	1	0
Renal failure and lung inflammation	0	0	0	0
Pituitary tumor	0	1	8	0
Pituitary and adrenal tumors	0	0	1	0
Brain tumor	0	1	1	0
Lung inflammation	0	1	0	0
Renal failure and pituitary cyst	0	1	0	0
Renal failure and arteries	0	0	2	0
Adrenal tumor	0	1	0	0
Pituitary and duodenal tumors	0	0	1	0

described above, renal disease alone or in combination with other disease processes was the probable cause of moribundity in more than 88% of the 13–18-month-old rats and in more than 58% of the 19–24-month-old rats (74% overall). Neoplasms accounted for moribundity in nearly 25% of the rats in the older group. Pituitary adenomas were associated with moribundity more than any other neoplasm.

Pathology

The results of histopathologic evaluation of all rats are presented in Table 3 (C-SS) and Table 4 (LS). These tables are divided into Neoplastic findings and Non-neoplastic findings and can be summarized as follows.

Urinary System

Kidney. — Chronic nephropathy, a common age-related lesion in rats (Figure 2 and Table 4), was diagnosed in all but one of the rats sacrificed in the LS (21–22 mo group), and in all but two of the rats 12 months of age or older in the C-SS. In 74% of the rats in the LS, this change was considered to be the primary cause of moribundity. Grossly, the kidneys were most often noted to be enlarged, discolored, and/or granular. Ascites, reflecting an inability to control fluid balance, was frequently present in severely affected rats. Microscopic changes were similar to those previously reported (Anver and Cohen, 1979). When tubular cysts were exceptionally large and noted at necropsy, the diagnosis of "cyst" was rendered in addition to the diagnosis of chronic nephropathy.

After chronic nephropathy, hydronephrosis was the most commonly observed renal lesion in both studies. The change was present in nearly 45% of the rats. In more than 90% of

Table 3. Cross-sectional Study, Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group

Diagnoses	6-Month $n = 15$	12-Month $n = 15$	18-Month $n = 20$	21-Month $n = 20$	24-Month $n = 20$	27-Month $n = 10$
Brain						
Hydrocephalus	0	0	0	1	0	0
Thalamus, mineralization	0	0	0	1	0	0
Cerebrum, astrocytoma	0	0	0	0	1	1
Mononuclear cell leukemia	0	0	0	0	0	1
Pons, granular cell tumor	0	0	0	0	0	1
Reticulosis	0	0	0	0	1	0
Thalamus, reticulosis	0	0	0	1	0	0
Spinal cord						
Axonal degeneration	0	0	2	1	0	0
Neuronal degeneration	0	0	1	0	0	0
Thyroids						
Arteritis	0	0	0	0	0	0
C Cell hyperplasia	0	0	0	1	2	1
Follicle, cyst	0	0	5	2	0	0
Follicular cell hyperplasia	0	0	2	3	1	1
Cyst	0	0	0	0	1	0
C Cell adenoma	0	0	0	0	0	1
Follicular cell adenoma	0	0	0	3	1	1
Follicular cell carcinoma	0	0	0	1	1	1
Parathyroids						
Cyst	0	0	0	0	0	1
Hyperplasia	0	0	2	9	5	3
Trachea						
Mineralization	0	0	0	0	1	0
Esophagus						
Salivary gland						
Fibrosis	0	0	0	0	1	0
Mandibular lymph node						
Cyst	0	0	1	3	0	0
Lymphoid hyperplasia	1	0	2	2	4	2
Plasmacytosis	0	5	4	5	3	3
Mononuclear cell leukemia	0	0	0	0	0	1
Pancreas						
Acinus, atrophy	7	8	14	13	10	5
Arteritis	0	0	3	7	4	5
Inflammation, chronic	0	1	0	0	0	0
Inflammation, chronic-active	1	0	0	0	1	0
Islet degeneration	0	0	0	0	1	0
Islet hyperplasia	0	1	0	0	0	0
Lymphoid infiltration	0	1	1	0	0	0
Acinus, hyperplasia	0	0	1	1	0	0
Islet cell adenoma	0	0	2	1	2	2
Islet cell carcinoma	0	0	0	1	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Acinus, adenoma	0	0	0	1	0	0
Thymus						
Atrophy	0	0	11	6	17	7
Congestion	1	0	0	0	0	0
Hemorrhage	5	6	2	0	1	0
Cyst	0	0	1	0	0	1
Mononuclear cell leukemia	0	0	0	0	0	1
Adrenal cortex						
Bilateral, congestion	0	0	0	0	1	0
Congestion	0	0	0	1	0	0
Cytoplasmic vacuolization	6	5	10	9	6	4
Degeneration	0	0	0	2	0	1
Hyperplasia	0	0	1	4	2	2
Hypertrophy	0	0	2	0	1	1
Thrombus	0	0	0	0	1	0

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Table 3. Cross-sectional Study, Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group *(Continued)*

Diagnoses	6-Month $n = 15$	12-Month $n = 15$	18-Month $n = 20$	21-Month $n = 20$	24-Month $n = 20$	27-Month $n = 10$
Adrenal cortex *(continued)*						
Arteritis	0	0	1	0	0	0
Adenoma	0	0	1	1	0	0
Mononuclear cell leukemia						
Adrenal medulla						
Hyperplasia	0	0	3	5	5	1
Mononuclear cell leukemia	0	0	0	0	0	1
Pheochromocytoma	0	0	0	4	2	1
Pituitary gland						
Pars distalis, hyperplasia	0	1	10	8	12	3
Pars distalis, cyst	0	0	0	1	1	0
Mononuclear cell leukemia	0	0	0	0	0	1
Pars distalis, adenoma	0	2	5	2	5	3
Heart						
Arteritis	1	0	0	1	1	1
Atrium, dilatation	0	0	0	0	0	1
Cardiomyopathy, chronic	0	4	8	7	9	5
Endocardium, hyperplasia	0	1	0	3	0	0
Fibrosis	0	0	0	0	1	2
Inflammation, chronic	0	0	0	0	1	0
Left atrium, thrombus	0	0	0	1	3	2
Lateral ventricle, endocardium, hyperplasia	0	0	0	0	3	0
Myocardium, hypertrophy	0	0	1	0	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Jejenum						
Mononuclear cell leukemia	0	0	0	0	0	1
Forestomach						
Hyperplasia	0	0	0	0	1	1
Inflammation, acute	0	0	0	0	0	1
Glandular stomach						
Inflammation, chronic	0	0	0	1	0	0
Necrosis	0	0	0	0	1	0
Glands, dilatation	0	0	1	0	0	0
Arteritis	0	0	0	1	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Skeletal muscle						
Degeneration	0	0	1	6	2	5
Thorax, fibrosis	0	0	0	0	1	0
Lipoma	0	0	0	0	2	0
Rhabdomyosarcoma	0	0	0	0	2	1
Liver						
Arteritis	0	0	1	1	0	0
Bile duct, hyperplasia	0	7	14	9	3	4
Clear cell focus	0	0	3	0	0	0
Cystic degeneration	0	1	5	3	6	1
Cytoplasmic vacuolization	4	2	2	0	0	0
Eosinophilic focus	0	2	2	5	5	4
Fatty change	0	0	0	0	0	2
Fibrosis	0	0	1	0	1	1
Hepatodiaphragmatic Nodule	0	0	0	0	1	0
Hepatocellular adenoma	0	0	0	0	1	0
Mononuclear cell leukemia	0	0	0	0	0	1
Spleen						
Developmental malformation	0	0	0	1	1	0
Hema cell proliferation	0	0	0	1	0	0
Hemangiosarcoma	0	0	1	0	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Lungs						
Alveolar epithelium, hyperplasia	0	0	1	1	1	0
Arteritis	0	0	1	0	0	0

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Table 3. Cross-sectional Study, Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group *(Continued)*

Diagnoses	6-Month $n = 15$	12-Month $n = 15$	18-Month $n = 20$	21-Month $n = 20$	24-Month $n = 20$	27-Month $n = 10$
Lungs *(continued)*						
Edema	0	0	0	0	0	1
Hemorrhage	2	1	1	0	0	0
Histiocytic infiltration	0	0	2	3	3	1
Inflammation, chronic-active	0	0	0	1	0	0
Inflammation, granulomatous	0	0	2	2	3	5
Interstitial inflammation	0	0	2	1	0	0
Mineralization	0	0	0	1	1	0
Lymphoid infiltration	0	0	10	5	3	2
Aveolar bronchiolar adenoma	0	0	0	1	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Kidneys						
Arteritis	0	0	0	0	1	0
Bilateral, hydronephrosis	0	0	2	0	1	0
Cyst	0	0	3	4	5	4
Lymphoid infiltration	1	0	0	0	0	0
Mineralization	0	0	1	0	0	0
Nephropathy, chronic	5	14	20	20	20	9
Pelvis, inflammation, acute	1	0	0	0	0	0
Renal papilla epith, hyperplasia	3	3	5	2	0	0
Right, hydronephrosis	11	7	11	14	10	6
Right, pelvis, mineralization	0	0	0	1	0	0
Right renal pelvis epith hyperplasia	3	2	1	2	3	0
Right, pelvis, inflam, acute	0	0	0	1	0	0
Right renal papilla epith hyperplasia	1	0	0	0	1	0
Mononuclear cell leukemia	0	0	0	0	0	1
Urinary bladder						
Duodenum						
Adenocarcinoma	0	0	0	0	1	0
Ileum						
Arteritis	0	0	1	0	0	0
Metazoan parasites	0	0	1	0	0	0
Cecum						
Arteritis	0	0	1	0	0	0
Metazoan parasites	0	0	2	0	1	0
Mononuclear cell leukemia	0	0	0	0	0	1
Colon						
Arteritis	0	0	1	0	0	0
Metazoan parasites	1	6	2	4	6	2
Eye						
Bilateral, cataract	0	0	0	7	4	3
Cataract	0	1	5	7	3	6
Ciliary body, histologic infiltration	0	0	11	12	10	10
Retina, atrophy	0	0	0	1	0	0
Testis						
Arteritis	0	0	1	0	1	0
Bilateral, atrophy	0	0	0	2	0	1
Degeneration	0	0	0	1	0	0
Epididymis						
Aspermia	0	0	1	0	0	0
Epith, cytoplasmic vacuolization	0	0	0	2	1	2
Prostate						
Atrophy	0	0	1	0	0	0
Hyperplasia	0	0	0	0	1	0
Inflammation, acute	0	1	0	0	0	0
Inflammation, suppurative	0	0	0	0	2	1
Lymphoid infiltration	1	0	0	0	0	0
Inflammation, granulomatous	0	0	0	1	0	0
Seminal vesicles						
Depletion	0	0	0	1	0	0

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Table 3. Cross-sectional Study, Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group *(Continued)*

Diagnoses	6-Month $n = 15$	12-Month $n = 15$	18-Month $n = 20$	21-Month $n = 20$	24-Month $n = 20$	27-Month $n = 10$
Seminal vesicles *(continued)*						
Dilatation	0	1	0	0	0	0
Hyposecretion	0	0	1	0	0	0
Preputial gland						
Abscess	1	1	0	0	1	0
Atrophy	0	0	0	1	1	2
Duct, dilatation	0	0	1	0	1	0
Inflammation, chronic	3	6	7	9	5	2
Inflammation, chronic-active	1	1	1	0	2	2
Inflammation, granulomatous	0	0	0	0	1	0
Nose						
Fungus	0	0	0	0	0	1
Inflammation, chronic-active	0	1	0	3	0	0
Nasolac duct, inflam, chronic	0	1	1	0	0	0
Olf epith, atrophy	0	0	2	3	0	0
Olf epith, cytopl alteration	0	0	0	1	0	0
Olf epith, degeneration	1	0	0	0	1	0
Olf epith, inflam, acute	0	2	0	0	0	0
Resp epith, foreign body	0	0	1	0	0	0
Resp epith, inflam, acute	1	0	1	0	0	0
Resp epith, inflam, chronic-active	0	0	1	1	5	2
Resp epith, inflam, subacute	0	1	2	0	3	1
Resp epith, inflam, suppurative	0	0	2	0	0	1
Inflammation, acute	0	0	0	1	0	0
Nasolac duct, inflam, chronic-active	0	0	0	1	0	0
Inflammation, subacute	0	0	0	0	0	1
Mononuclear cell leukemia	0	0	0	0	0	1
Tympanic bullae						
Mononuclear cell leukemia	0	0	0	0	0	1
External ear						
Sternum (bone)						
Mononuclear cell leukemia	0	0	0	0	0	1
Bone marrow						
Hyperplasia	0	0	0	1	0	0
Mononuclear cell leukemia	0	0	0	0	0	1
Cauda equina						
Axonal degeneration	0	0	12	15	14	9
Inflammation, granulomatous	0	0	0	1	1	3
Mononuclear cell leukemia	0	0	0	0	0	1
Other tissues and lesions:						
Aorta-normal	0	0	0	0	1	0
Bone, forefoot, hyperostosis	0	0	0	0	1	0
Bone, hindfoot, abscess	0	0	0	0	1	0
Bone, hindfoot, hyperostosis	0	0	0	0	1	0
Lymph node (Ln), lumbar-hemorrhage	0	0	1	1	0	0
Ln, mediastinal-hemorrhage	0	0	0	3	1	0
Ln, mes-hemorrhage	0	2	1	0	2	1
Ln, mes-lymphoid hyperplasia	0	0	0	0	0	1
Ln, mes-plasmacytosis	0	1	0	1	0	0
Mammary gland-lactation	0	0	0	0	1	1
Mesentery-arteritis	0	0	2	5	2	2
Mesentery-inflam, chronic	0	0	0	1	0	0
Skin-normal	0	0	0	0	1	1
Skin-subcutis-fibrosis	0	0	0	0	1	0
Skin-tail-abscess	0	0	0	3	1	0
Skin-tail-hyperkeratosis	0	0	0	0	1	0
Skin-tail-inflam, chronic-active	0	0	0	0	0	1
Skin-tail-inflam, suppurative	0	0	0	0	1	1
Skin-tail-sq hyperplasia	0	0	0	0	1	0
Skin-tail-ulcer	0	0	0	0	1	0
Spinal nerve-axonal, degeneration	0	0	3	6	4	0
Spinal nerve-inflam, granulomatous	0	0	0	1	0	0
Tooth-abscess	0	0	0	1	0	0

Table 4. Longitudinal Study; Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group

Diagnoses	13–18 Month $n = 52$	19–20 Month $n = 32$	21–22 Month $n = 14$	23–24 Month $n = 2$
Brain				
Hydrocephalus	0	0	1	0
Developmental malformation	0	1	0	0
Cerebellum, granular cell tumor	0	1	0	0
Cerebrum, astrocytoma	2	0	1	0
Spinal cord				
Neuronal degeneration	1	0	0	0
Thyroids				
Arteritis	2	0	0	0
C Cell hyperplasia	1	2	0	0
Follicle, cyst	4	2	2	0
Follicular cell hyperplasia	3	3	2	0
Cyst, squamous	1	0	0	0
Follicular cell adenoma	3	1	1	0
Follicular cell carcinoma	1	1	0	0
Parathyroids				
Atrophy	0	1	0	0
Hyperplasia	35	13	5	0
Trachea				
Arteritis	1	0	0	0
Esophagus				
Salivary gland				
Edema	2	0	1	0
Inflammation, acute	1	0	0	0
Lymphoid infiltration	1	0	0	0
Sarcoma, not otherwise specified	1	0	0	0
Mandibular lymph node				
Cyst	1	0	0	0
Lymphoid hyperplasia	11	7	3	0
Plasmacytosis	5	2	0	0
Edema	3	2	2	1
Hemorrhage	1	0	1	0
Pancreas				
Arteritis	34	13	2	0
Acinus, atrophy	19	14	6	1
Acinus, hyperplasia	1	1	0	0
Edema	1	0	0	0
Islet cell adenoma	0	1	0	0
Acinus, adenoma	1	0	0	0
Thymus				
Atrophy	38	21	8	1
Amyloid/paramyloid	1	0	0	0
Adrenal cortex				
Accessory structure	2	1	0	0
Congestion	0	1	0	0
Cytoplasmic vacuolization	19	14	5	0
Degeneration	1	3	0	1
Hyperplasia	10	4	3	0
Hypertrophy	8	11	3	0
Hemorrhage	3	1	0	0
Arteritis	1	0	0	0
Bilateral, hemorrhage	0	2	0	0
Bilateral hypertrophy	0	2	0	0
Carcinoma	1	0	0	0
Adrenal medulla				
Hyperplasia	25	11	3	0
Arteritis	1	0	0	0
Pheochromocytoma	3	4	0	1
Bilateral, pheochromocytoma	0	1	1	0
Pituitary gland				
Pars distalis, hyperplasia	19	11	2	2
Pars distalis, cyst	1	0	0	0
Pars distalis, angiectasis	1	0	0	0
Pars intermedia, hyperplasia	1	0	0	0
Pituitary gland *(continued)*				
Pars distalis, adenoma multiple	0	0	1	1
Pars distalis, adenoma	3	8	7	0
Heart				
Arteritis				
Left atrium, dilation	3	0	0	1
Cardiomyopathy, chronic	26	14	7	1
Left atrium, thrombus	5	3	1	1
Left ventricle, dilation	0	1	0	0
Mineralization	1	0	0	0
Rt ventricle, dilation	1	0	0	0
Endocardial hyperplasia	1	0	0	0
Jejunum				
Arteritis	3	0	0	0
Adenocarcinoma	0	1	0	0
Forestomach				
Arteritis	1	0	0	0
Edema	1	0	0	0
Epith degeneration	1	0	0	0
Squamous hyperplasia	3	0	0	0
Ulcer	7	1	0	0
Ulcer, chronic	0	0	1	0
Inflammation, subacute	1	0	0	0
Hyperplasia	1	0	0	0
Squamous papilloma	1	0	0	0
Glandular stomach				
Inflammation, acute	1	0	0	0
Necrosis	1	0	0	0
Arteritis	13	1	0	0
Edema	2	0	0	0
Mineralization	2	0	0	0
Hyperplasia	1	0	0	0
Skeletal muscle				
Degeneration	3	0	2	0
Fibroma	1	0	0	0
Liver				
Arteritis	9	3	0	0
Bile duct, hyperplasia	12	17	9	1
Clear cell focus	1	0	0	0
Cystic degeneration	10	10	6	0
Eosinophilic focus	8	6	3	0
Fatty change	2	1	0	0
Hepatodiaphragmatic nodule	3	1	1	0
Developmental malformation	1	0	0	0
Basophilic focus	1	1	0	0
Eosin focus, multiple	0	1	0	0
Spleen				
Hema cell proliferation	13	2	0	0
Hemosiderosis	0	0	1	0
Atrophy	1	0	0	0
Fibrosis	1	1	0	0
Arteritis	1	2	0	0
Developmental malformation	0	1	0	0
Lungs				
Alveolar epithelium, hyperplasia	1	0	2	1
Alveolar macrophages	1	0	0	0
Arteritis	6	2	0	0
Inflammation, chronic	6	2	0	0
Inflammation, granulomatous	9	8	2	0
Lymphoid infiltration	4	4	6	0
Osseous metaplasia	1	0	0	0
Congestion	1	0	0	0

(Continues next page)

Table 4. Longitudinal Study; Incidence of Neoplastic and Non-Neoplastic Microscopic Findings by Age Group *(Continued)*

Diagnoses	13–18 Month $n = 52$	19–20 Month $n = 32$	21–22 Month $n = 14$	23–24 Month $n = 2$
Lungs *(continued)*				
Mineralization	1	0	0	0
Pigment	0	1	0	0
Kidneys				
Bilateral, hydronephrosis	5	3	0	0
Cyst	15	5	3	0
Hemorrhage	0	0	1	0
Nephropathy, chronic	52	32	13	2
Right, hydronephrosis	35	17	9	1
Right cyst	0	0	1	0
Renal pelvis epith, hyperpl	2	1	0	0
Hemangiosarcoma	1	0	0	0
Urinary bladder				
Duodenum				
Arteritis	2	0	0	0
Hyperplasia	1	0	0	0
Inflammation, chronic	1	1	0	0
Leiomysosarcoma	0	0	1	0
Ileum				
Arteritis	19	1	0	0
Inflammation, chronic	1	1	0	0
Lymphoid hyperplasia	1	0	0	0
Leiomyosarcoma	0	0	1	0
Cecum				
Arteritis	14	0	0	0
Metazoan parasites	2	1	0	0
Colon				
Arteritis	14	0	0	0
Metazoan parasites	2	1	0	0
Inflammation, acute	1	0	0	0
Eye				
Cataract	0	0	1	0
Ciliary body, hist cell infilt	24	20	8	2
Lens, cataract	7	6	3	0
Bilateral, lens, cataract	1	4	2	1
Cornea, ulcer	0	1	0	0
Retina, degeneration	0	0	2	1
Testis				
Arteritis	5	1	0	0
Atrophy	0	0	1	0
Bilateral, atrophy	1	0	0	0
Degeneration	1	0	0	0
Hypospermia	1	0	0	0
Bilateral, hypospermia	4	0	0	0
Epididymis				
Hypospermia	5	0	0	0
Duct epith, cytoplasmic Vacuolization	3	0	0	0
Prostate				
Atrophy	5	3	1	0
Dilation	2	0	0	0
Inflammation, acute	2	0	0	0
Arteritis	1	1	0	0
Inflammation, subacute	1	0	0	0
Seminal vesicles				
Depletion	1	0	0	0
Dilatation	2	0	0	0
Hyposecretion	1	0	0	0
Preputial gland				
Abscess	3	0	0	0
Atrophy	5	2	0	0
Duct, dilatation	3	3	1	0
Inflammation, chronic	17	11	3	2
Inflammation, granulomatous	1	0	0	0
Inflammation, subacute	1	1	0	0
Bilateral, duct, dilatation	0	0	1	0

Diagnoses	13–18 Month $n = 52$	19–20 Month $n = 32$	21–22 Month $n = 14$	23–24 Month $n = 2$
Nose				
Fungus	0	1	0	0
Inflammation, chronic-active	16	10	1	0
Goblet cell hyperplasia	3	0	0	0
Olf epith, atrophy	4	1	0	0
Olf epith, cytopl alteration	2	0	0	1
Olf epith, degeneration	2	0	0	0
Inflammation, subacute	6	3	1	0
Inflammation, chronic	3	1	1	0
Inflammation, acute	1	0	1	0
Hemorrhage	1	0	0	0
Inflammation, suppurative	0	0	1	0
Maxillary sinus, inflam, chronic	1	0	0	0
Submucosal glands, hyperplasia	0	1	0	0
Tympanic bullae				
External ear				
Schwannoma	0	0	1	0
Sternum (bone)				
Bone marrow				
Myeloid atrophy	1	0	0	0
Myelofibrosis	1	0	0	0
Cauda equina				
Axonal degeneration	19	19	11	2
Other tissues and lesions:				
Aorta-mineralization	2	0	0	0
Bone, cranium-develop malformation	0	1	0	0
Aorta, arteritis	1	0	0	0
Foot-inflam, chr-act	1	0	0	0
Lymph node (Ln), mes-lymphoreticular hyperplasia	1	0	0	0
Ln, lumbar-hemorrhage	1	0	0	0
Ln, renal-edema	1	0	0	0
Ln, mediastinal-hemorrhage	11	3	1	0
Ln, mes-hist cell edema	1	0	0	0
Ln, media-hist cell edema	1	0	0	0
Ln, media-lymph hyperpl	1	0	0	0
Mesentery-arteritis	26	2	0	0
Mammary gland-hyperplasia	0	0	1	0
Nerve, cranial-axonal degen	1	0	0	0
Skin-epithelial incl cyst	0	1	0	0
Skin-abscess	0	1	0	0
Skin, tail-abscess	0	1	1	0
Skin, tail-hyperkeratosis	1	0	0	0
Skin, tail-inflam, chr-act	1	0	0	0
Skin, tail-parakeratosis	1	0	0	0
Skin, foot ulcer	1	0	0	0
Spinal nerve-axonal, degen	6	0	1	0
Skin, adnexa-atrophy	1	0	0	0
Tail, abscess	1	0	0	0
Skin, foot-acanthosis	0	1	0	0
Skin, foot-hyperkeratosis	0	1	0	0
Pharynx, palate-sq papilloma	1	0	0	0
Skin-fibroma	1	0	1	0
Skin-keratoacanthoma	2	1	1	0
Skin, subcutis-fibroma	2	1	1	0
Skin, subcutis-fibrosarc	1	0	1	0
Skin, squamous papilloma	0	1	0	0
Skin, keratoacanthoma, mult	0	1	0	0
Tooth-odontoma	0	1	0	0
Zymbal's gland-carcinoma	0	2	0	0

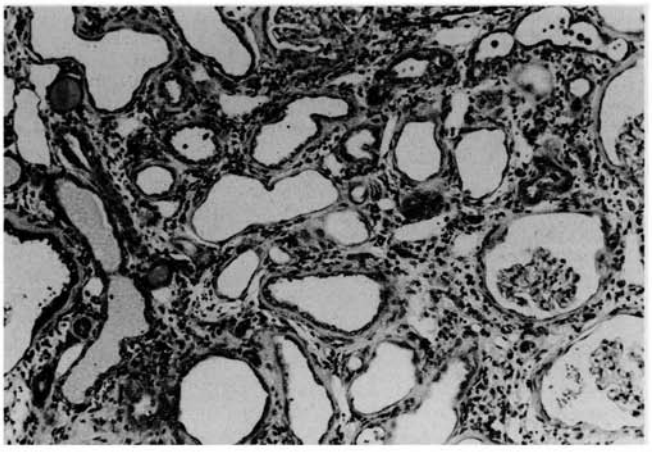

Figure 2. Kidney. Chronic nephropathy with multiple tubular dilations, interstitial fibrosis with lymphocytic infiltrates. H&E, 115.5×.

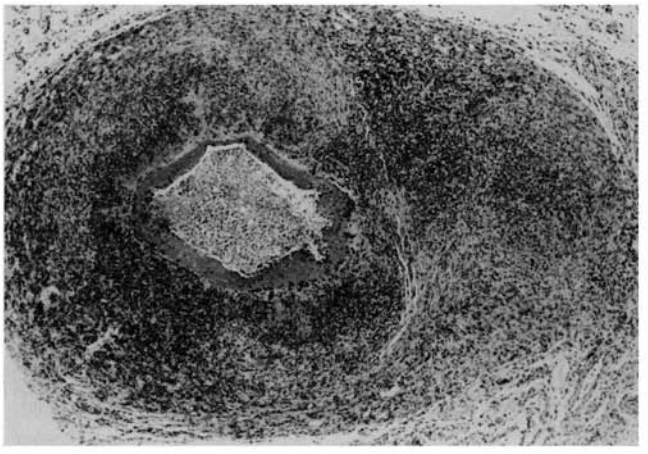

Figure 3. Mesenteric artery. Arteritis with medial fibrinoid necrosis, periarterial fibrosis, and a mixed inflammatory cell infiltrate. H&E, 46.2×.

the rats with hydronephrosis, only the right kidney was affected. In the remaining rats with this change, the lesion was bilateral. Severity ranged from minimal (slight dilatation of the renal pelvis) to severe (enlarged cystic kidney with a thin rim of renal tissue). Severe changes were noted in only two rats, however, and both were in the 6-month age group. Focal or multifocal hyperplasia of the epithelium lining the renal papilla and pelvis was present in rats with hydronephrosis. A relatively high incidence of spontaneous hydronephrosis of the right kidney in Wistar-related male rats has been previously reported (O'Donoghue and Wilson, 1977). No definitive cause has been associated with this finding. Hydronephrosis in some rat strains has been reported as a congenital rather than an acquired age-related lesion (Cohen et al., 1970).

Urinary bladder. — No neoplastic or significant nonneoplastic lesions were noted in the urinary bladder.

Figure 4. Heart. Chronic cardiomyopathy with degeneration of the myofibers, fibrosis, and a mononuclear cell infiltrate. H&E, 115.5×.

Cardiovascular System

Blood vessels. — Arteritis (Figure 3) was the most commonly diagnosed change involving the cardiovascular system (Anver and Cohen, 1979). While numerous tissues were affected by this change, lesions were most severe in the mesenteric and pancreatic arteries. In the C-SS, arteritis was not diagnosed in rats less than 18 months old.

Heart. — Chronic cardiomyopathy (Figure 4) was a common finding in rats over 6 months of age in both studies (MacKenzie and Alison, 1990). The left ventricle was more frequently and severely involved; however, the apex, interventricular septum, and papillary muscles were often affected as well. In several rats 24 months of age or older, fibrosis was diagnosed in lieu of chronic cardiomyopathy as there was no longer any indication of myofibrial degeneration.

Large organizing thrombi were present in the left atria of six rats 21 months of age or older in the C-SS, and in nine rats in the LS. Affected atria were usually noted at necropsy to be markedly dilated. In two of the rats in the LS, the thrombi were so large that they were noted to be at least partially responsible for the moribund condition of the rat. Left atrial dilatation without thrombi was noted in three rats in the LS.

Endocardial hyperplasia (Figure 5) was present in seven rats in the C-SS and in three rats in the LS. This lesion was usually more prominent in the left ventricle, although in three rats the change was limited to the left atrium (Anver and Cohen, 1979; MacKenzie and Alison, 1990).

Respiratory System

Nose. — A variety of inflammatory lesions was present in the nose of rats from both study groups. The inflammation ranged from small foci of polymorphonuclear cells or lymphocytes to extensive infiltrations of mixed inflammatory cells involving several areas of the mucosa, nasal cavity, and nasolacrimal duct. Based upon the incidence of inflammation in the various cross-sectional groups, no relationship to age

Figure 5. Heart. Endocardial hyperplasia characterized by proliferation of undifferentiated mesenchymal cells. H&E, 115.5×.

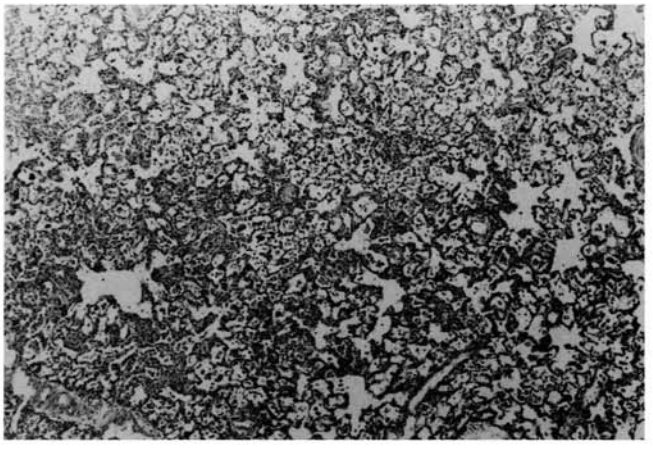

Figure 6. Lung. Aggregations of histiocytes are present in the alveolar spaces. H&E, 46.2×.

was apparent. Infectious agents such as SDAV (Jacoby, 1986) and inhaled material such as bedding material or ammonia (from bacterial breakdown of urine) were the likely cause of many of the inflammatory changes. Fungal hyphae were present in the nasal cavity of one of the rats.

Several alterations of the olfactory epithelium were noted. These included loss of olfactory and sustentacular cells resulting in a thinner olfactory mucosa and/or degeneration of these cells characterized by the presence of brightly eosinophilic cytoplasmic granules. While these granules are not an uncommon finding in rat olfactory epithelium, the cause of this change is unknown.

Lungs. — A variety of non-neoplastic lesions were observed in the lungs, but few were thought to be age-related. Lymphoid infiltration into the periarteriolar interstitial tissue was the most frequently diagnosed change in the C-SS and the second most frequently diagnosed change in the LS. Although this change was not noted in C-SS rats less than 18 months of age, an age-associated increase was not apparent. This change is probably associated with infection with PVM (Richter, 1986). Granulomatous inflammation and histiocytic infiltration were two similar conditions which, if considered together, were the most commonly diagnosed lesions in the lungs of rats in both studies. Aggregations of histiocytes (alveolar macrophages) were seen in rats greater than 18 months of age in the C-SS and with a slightly greater incidence in rats over 19 months of age in the LS. In the latter study, the macrophage aggregates were most often more numerous, larger, and included multinucleated cells and lymphocytes (Figure 6). The diagnosis of granulomatous inflammation was given to these lesions.

One primary lung tumor, an alveolar/bronchiolar adenoma, was present in one 21-month-old rat in the C-SS. Spontaneous tumors of the respiratory tract of rats have been reported in the literature, but their incidence is low (Pour et al., 1976; Squire and Goodman, 1978). Other incidental findings included focal mineralization of the pulmonary arteries, osseous metaplasia, and alveolar epithelial hyperplasia.

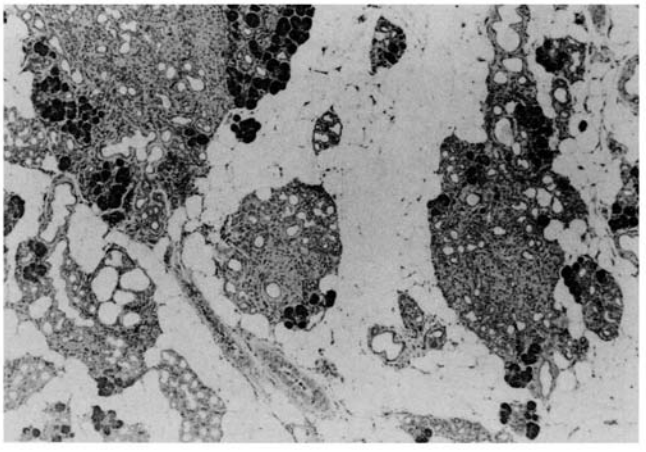

Figure 7. Pancreas. Atrophy of pancreatic acinar cells. H&E, 46.2×.

Trachea. — Mineralization of the tracheal cartilage was diagnosed in two rats over 21 months of age.

Alimentary System

Pancreas (Exocrine). — Focal or multifocal atrophy of the pancreatic acinar cells was the most frequently diagnosed lesion in this organ (Figure 7). In severe cases, there was nearly a complete loss of acinar cells. Despite the loss of acinar cells, the pancreatic islets appeared normal even in severely atrophic pancreata. While this has been reported to be the most common age-associated lesion in some strains of rats (Burek, 1978), in the C-SS no relationship between age and incidence or severity was noted.

Non-neoplastic and neoplastic proliferations of acinar cells were present in both studies. Acinar cell hyperplasia was diagnosed in two rats of each study. No relationship to age could be established. In addition, one acinar cell adenoma was diagnosed in each study.

Liver. — Nearly identical incidences of bile duct hyperplasia were noted in the C-SS (37%) and LS (39%) studies

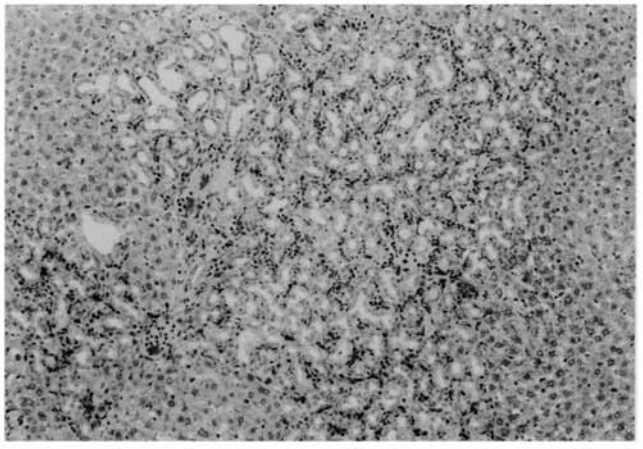

Figure 8. Liver. Hyperplasia of the bile ducts accompanied by a periductal mononuclear cell infiltrate. H&E, 115.5×.

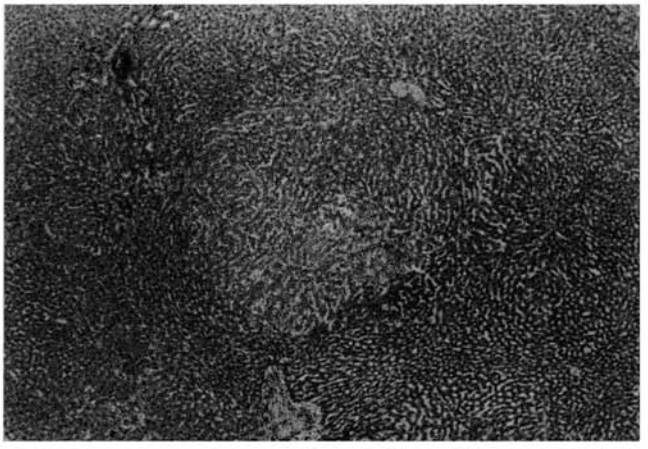

Figure 10. Liver. Eosinophilic focus. H&E, 46.2×.

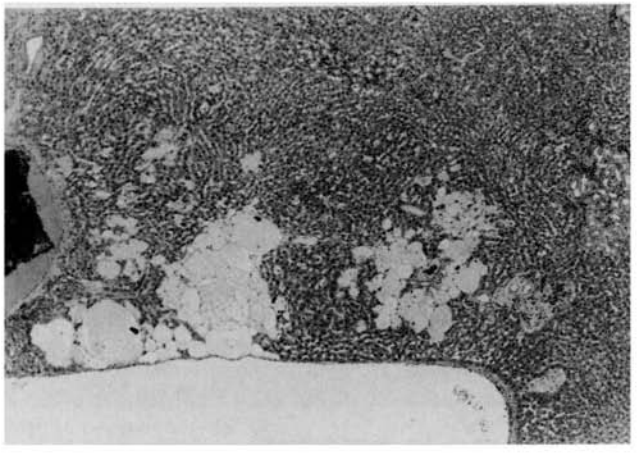

Figure 9. Liver. Cystic degeneration. Spaces contain an eosinophilic fibrillar material. H&E, 46.2×.

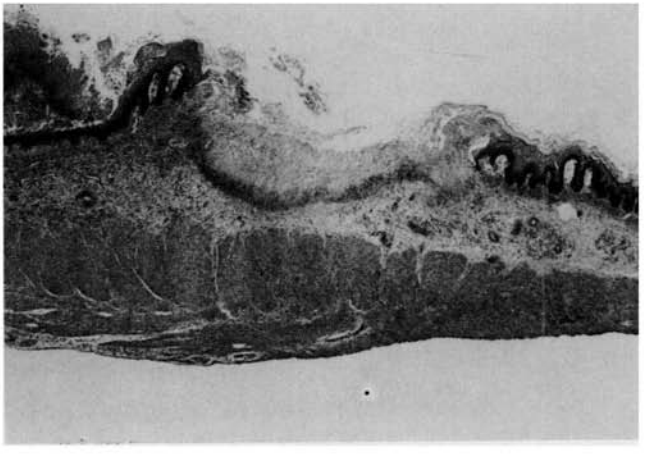

Figure 11. Forestomach. Focal ulceration accompanied by submucosal edema and an inflammatory cell infiltrate. H&E, 23.1×.

(Figure 8). In the C-SS, no relationship to age was apparent. In the LS, however, the incidence of bile duct hyperplasia in rats 19–24 months was more than twice that of rats 13–18 months old (56% vs 23%).

Focal or multifocal cystic degeneration (Figure 9) was (present in the livers of 16% of rats in the C-SS compared to 26% of those in the LS. While the sizes varied considerably from that of a few hepatocytes to more than 10 times that size, the morphology of the structures varied little. As with bile duct hyperplasia, little relationship to age could be established in the C-SS, although in the LS the incidence in the 19–24-month-old rats (33%) was considerably greater than that among 13–18-month-old rats (19%). It is thought that these structures represent degenerative hepatocytes (Anver and Cohen, 1979).

Foci of cellular alteration were diagnosed when focal tinctorial and/or morphologic changes in hepatocytes set them apart from the surrounding parenchyma (Stewart et al., 1980). The foci seen in these two studies were of three types: eosinophilic, basophilic, and clear cell. Eosinophilic foci (Figure 10) were the most frequently seen and were more numerous in the old rats in both studies. The incidences of clear cell and basophilic foci were too small to establish an age relationship.

Hepatodiaphragmatic nodules were noted in five rats from the LS and one from the C-SS. These nodules, occurring on the diaphragmatic surface of the liver (usually median lobe), are not uncommon findings in many strains of rats and are thought to be due to a focal weakening of the liver capsule allowing parenchyma to protrude (Anderson, 1949).

One primary liver tumor was noted. An hepatocellular adenoma was present in a 24-month-old rat in the C-SS.

Stomach. — Ulceration of the forestomach (nonglandular stomach) was noted in nine rats in the LS (Figure 11). In most of these, the ulcers were quite deep, extending through the submucosa into the tunica muscularis. Despite the severe inflammation accompanying the ulcers, glandular stomachs of affected rats displayed little or no inflammatory change.

A squamous papilloma was noted in one rat in the LS,

while hyperplasia of the squamous epithelium was diagnosed in several rats from both studies.

Intestines. — Several malignant tumors were present in the small intestines. An adenocarcinoma was present in the duodenum of one rat in the C-SS and in the jejunum of one rat in the LS. In addition, one leiomyosarcoma was diagnosed in the duodenum of one rat in the LS. No tumors were noted in the large intestines of any of the rats.

Oral cavity. — Only three lesions were noted in the oral cavity of rats from either study. A squamous papilloma was present on the palate of one rat in the LS, and two lesions of the teeth were diagnosed. In the LS, an odontoma was present in one rat, and in the C-SS, a tooth abscess was diagnosed.

Salivary gland. — Spontaneous neoplastic and non-neoplastic lesions of the salivary gland are reported to be rare in rats (Squire and Goodman, 1978). This was found to be the case in these studies as well. Only one salivary gland neoplasm (sarcoma, NOS) was diagnosed.

Endocrine System

Thyroid glands. — Non-neoplastic lesions of the thyroid glands were relatively infrequent and insignificant. In both studies they consisted of follicular cysts, follicular cell hyperplasia, C-cell hyperplasia, and squamous cysts. A modest increase in interfollicular connective tissue was noted in some older rats, but this change was not diagnosed separately. Follicular cysts were seen in 7% of rats from the C-SS and 8% of the LS. Although none were diagnosed in C-SS among rats less than 18 months of age, no age relationship could be discerned in either study. The other thyroid cysts which were diagnosed (one in each study) were small solitary structures lined by squamous epithelium. These cysts (ultimobrancial cysts) are developmental remnants and are not uncommon findings in rats of all ages (Anver and Cohen, 1979).

Focal follicular cell hyperplasia was noted in rats of both studies (7% in the C-SS and 8% in the LS), and like the follicular cysts, no clear relationship to age was noted (although none was present in cross-sectional rats less than 18 months of age).

Hyperplasia of C (parafollicular) cells was noted in five rats from the C-SS and three rats from the LS. While no C-SS rats under 18 months of age had this change, there was no age-related increase in incidence of the change in the other groups.

Three types of neoplasms were noted in the thyroid glands. Follicular cell tumors were diagnosed in eight rats from the C-SS 21 months of age or older and in seven rats from the LS (both age groups). Ten were adenomas and five were carcinomas. No distant metastases for the carcinomas were noted. One C cell adenoma was noted (27-month group, C-SS).

Parathyroid glands. — Changes in the parathyroid glands were common in both studies and consisted almost exclusively of hyperplasia. In some cases, the parathyroid glands were larger than the thyroid glands in the histologic section (Figure 12). These changes were not unexpected findings in light of the severe renal disease present in most of the rats, since renal secondary hyperparathyroidism is a common finding in animals with chronic renal disease (Anver and Cohen, 1979).

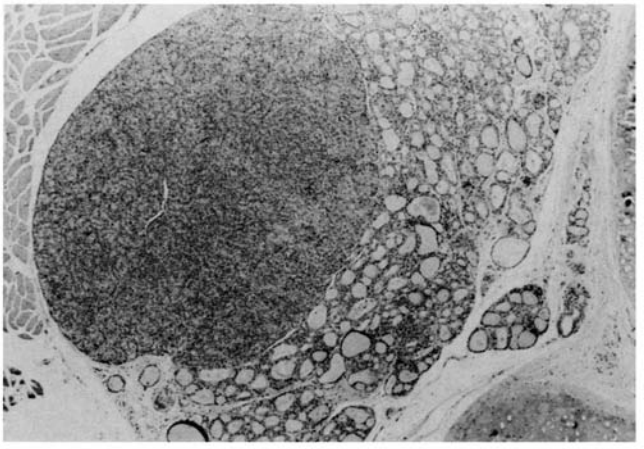

Figure 12. Parathyroid. The gland in hyperplastic. H&E, 46.2 ×.

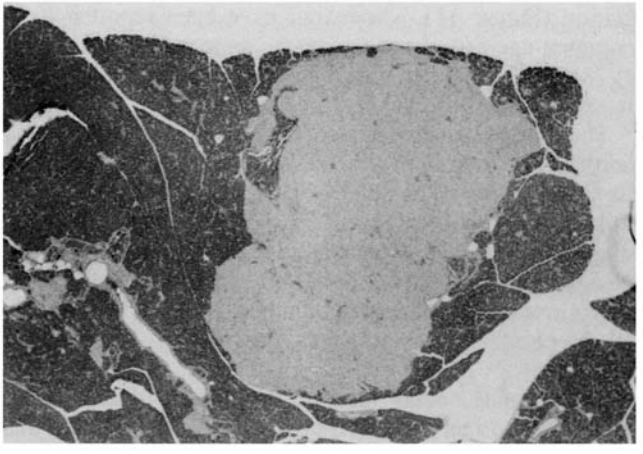

Figure 13. Pancreas. Islet cell adenoma. H&E, 23.1 ×.

Pancreas (islets). — Lesions of the pancreatic islets were almost exclusively proliferative (one rat had focal degeneration), and all but one of the proliferative lesions were neoplastic. Islet cell adenomas (Figure 13) were present in the C-SS among 7 rats 18 months of age or older and in one rat from the LS. In addition, one islet cell carcinoma was present in the C-SS among rats 18 months of age or older and in one rat from LS. In addition, one islet cell carcinoma was present in the C-SS.

Adrenal glands. — Numerous non-neoplastic lesions of the adrenal cortex were present in rats of both studies. The lesion most frequently seen was focal cytoplasmic vacuol-

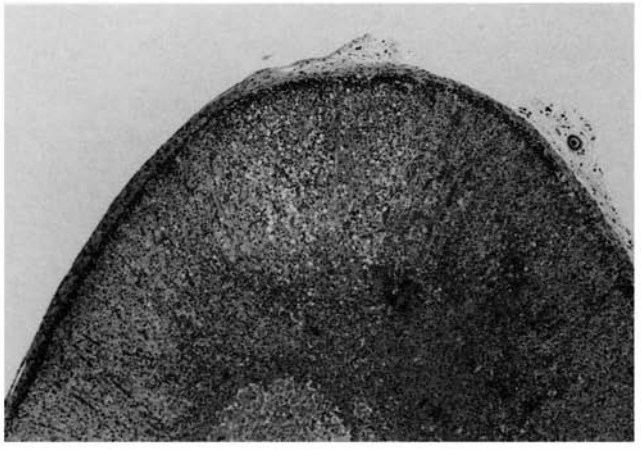

Figure 14. Adrenal. There is focal cytoplasmic vacuolization in the cortex. H&E, 46.2×.

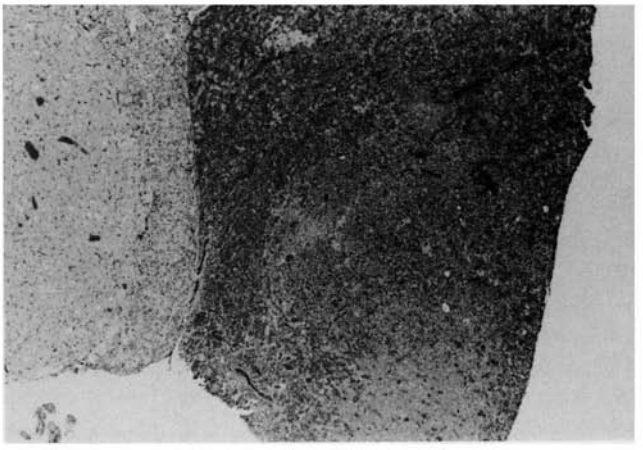

Figure 15. Pituitary gland. Focal hyperplasia of the pas distalis. H&E, 46.2×.

ization (Figure 14). These foci have been reported to be common age-associated lesions in some rat strains (Burek, 1978), but in neither the C-SS nor the LS was there any increase in age-related incidence.

Focal hyperplasia was also a relatively common adrenal cortical lesion in both studies. In other reports, these foci have been grouped together with vacuolated cells and found to be age-related (Burek, 1978). While this lesion was not diagnosed in rats less than 18 months of age in the C-SS, no age-related increase existed in the incidence of the lesion in rats greater than 18 months. In the LS, the incidence in the 19–24-month groups was less than in the 13–18-month group.

Hypertrophy, or an increase in the size of the adrenal cortex due to increased size of constituent cells rather than their number, was noted with much greater frequency in the LS than in the C-SS. In addition, the 19–24-month groups had a much greater incidence than the 13–18-month group. In most cases the change was focal. In some cases, however, the change was diffuse. Given the physiology of the adrenal cortex, it is likely that the increased incidence in the moribund animals was associated with stress associated with the disease conditions in these animals (Jones and Hunt, 1983). Neoplastic lesions of the adrenal cortex consisted of two adenomas in the middle-aged groups (18 months and 21 months) in the C-SS and one carcinoma in the LS.

Primary lesions of the adrenal medulla were solely proliferative in nature in both studies. Overall, focal hyperplasia was diagnosed in 14% of the C-SS rats, all of which were 18 months of age or older, and in 39% of the LS rats. Pheochromocytomas showed a definite age association in that all C-SS rats with this lesion were 21 months of age or older, and the incidence in the 19–24-month groups was markedly higher than the incidence in the 13–18-month group in the LS (16% vs 6%). A previous report (Gillman et al., 1953) indicated that the incidence of pheochromocytomas in Wistar rats older than 24 months was greater than 76%. However, these authors included foci of hyperplasia in their definition of pheochromocytomas. Conversely, an even earlier study of adrenal glands from 15 male and 16 female Wistar albino rats and 3 male and 3 female Norway rats reported the incidence of focal hyperplasia to be 11% in female and 51% in male rats. The later study excluded the diagnosis of pheochromocytoma even though it was acknowledged that "when arranged in order of size they [degree of hyperplasia] suggested the development of an adenoma" (Yeakel, 1947).

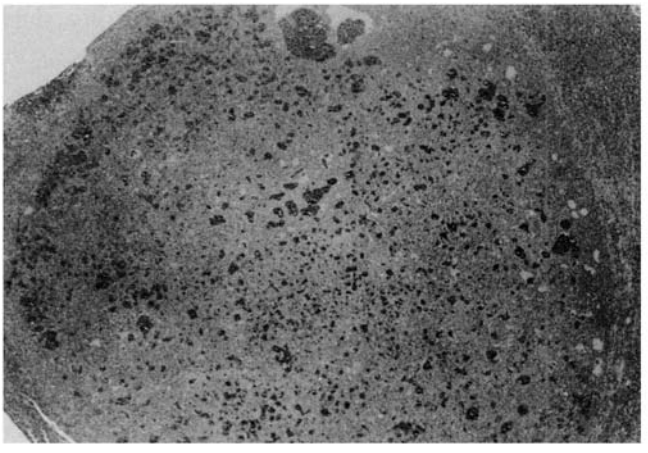

Figure 16. Pituitary gland. Adenoma of the pars distalis. H&E, 23.1×.

Pituitary gland. — Other than hyperplasia of the pars distalis, non-neoplastic lesions of the pituitary gland were very infrequent and insignificant. Focal or multifocal hyperplasia of the pars distalis (Figure 15) was diagnosed in more than 47% of the C-SS at 18 months or older, but in only 7% of the 12-month-old rats and in no 6-month-old rats. Thirty-four percent of rats from the LS had focal or multifocal hyperplasia.

One or more pituitary adenomas (Figure 16) were present in 17% of the C-SS and in 20% of rats in the LS. None was seen in the C-SS among rats less than 12 months of age, and the incidence varied from 10% in the 21-month group to 30% in the 27-month group. In the LS the difference in incidence between the two groups was striking. The inci-

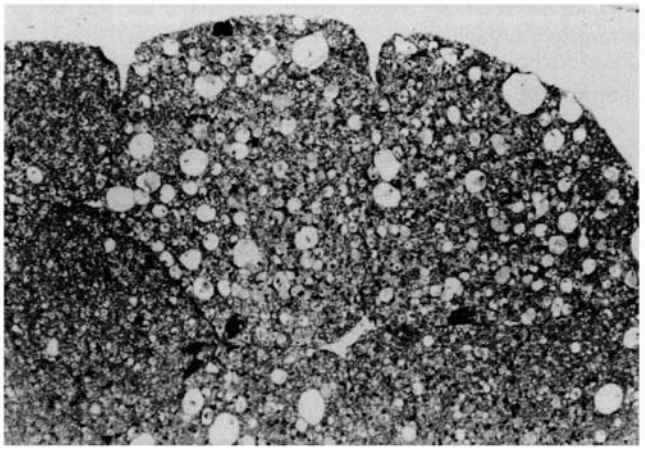

Figure 17. Cauda equina. Axonal degeneration accompanied by lipid-laden macrophages. H&E, 115.5×.

dence in the 13–18-month groups was only 6%, whereas the incidence in the 19–24-month groups was 34%.

Nervous System

Brain. — Only a few spontaneous non-neoplastic lesions were present in the brain. These included hydrocephalus (one per study), reticulosis, and mineralization. Neoplastic lesions consisted of astrocytomas (two in rats 24 months of age or greater in the C-SS and three in the LS) and granular cell tumors (one per study).

Spinal cord (including cauda equina). — Lesions of the spinal cord and cauda equina were limited primarily to axonal degeneration in the cauda equina (Figure 17). This change was also seen in other parts of the spinal cord and in spinal nerves as well, but to a lesser degree. In the C-SS, the lesion was present only in rats 18 months of age or older. The incidence in the oldest age group was 90%. In the LS, the incidence in the 19–24-month groups was far greater than that in the 13–18-month dose group.

Reproductive System

Testes. — Relatively few lesions were noted in the testes, none of which were neoplastic. Testicular atrophy, either unilateral or bilateral, was diagnosed in the C-SS among six rats 18 months or older, but in only two rats in the LS. The change was characterized by loss of spermatozoa and seminiferous cells to a greater or lesser degree. Degenerative seminiferous tubules were formed in one rat in each study. Hypospermia was diagnosed in five rats in the LS.

Epididymis. — Hypospermia and aspermia were observed in the epididymis of several rats. These findings, however, actually reflect changes occurring in the testes (Roberts, 1971). The only other change diagnosed in the epididymides was cytoplasmic vacuolization of the epithelial cells lining the ductus epididymis. This alteration was present only when there was a lack of spermatozoa in the ductus due to testicular atrophy or degeneration. The etiology of the change is unknown, but it is thought to be due to a lack of physiologic interaction with or stimulation by the spermatozoa which are normally present (Roberts, 1971).

Prostate gland. — Atrophy, characterized by a moderate to marked reduction in size of glandular epithelial cells and a decrease in or absence of glandular secretion within the lumina, was noted in nine rats from the LS but in only one rat from the C-SS. No relationship to age could be identified. Various types of inflammation, from acute to granulomatous, were diagnosed infrequently in both studies. Inflammatory cellular infiltrates were seen in rats as young as 6 months of age. Glandular lumina contained inflammatory cells and necrotic debris in four rats in the C-SS and in two from the LS diagnosed with acute or suppurative inflammation. In others, only the interstitium was involved. No relationship to age could be noted in these studies.

Seminal vesicles. — Very few lesions were noted in the seminal vesicles, and all were related to the amount of secretion within the glandular lumina. Due to a normal physiologic variance in the amount of secretion present, and because of the small number of lesions noted, no relationship to age could be noted.

Preputial gland. — A large number of inflammatory lesions were diagnosed in the preputial glands of rats from both studies. These ranged from acute inflammatory cell infiltrations to chronic inflammation with abundant fibrosis. Inflammation was more frequent in older rats. However, rats as young as 6 months had various types of inflammatory lesions.

Musculoskeletal System

Skeletal muscle. — The only change noted in the routine section of muscle was myofiber degeneration. These changes were normally minimal or mild and were likely associated with the degenerative axons described above (Van Steenis and Kroes, 1971). In the C-SS there was a definite relationship to age. Of the 14 rats with this change, only one was less than 21 months of age.

A small number of miscellaneous lesions were diagnosed in the skeletal muscle as a result of histologic examination of gross lesions noted at necropsy. Most of these were neoplastic lesions and included two lipomas (both in 24-month-old rats from the C-SS), one rhabdomyosarcoma in a 27-month-old rat, and one fibroma in the LS.

Bone. — No lesions were diagnosed in the sternum. Several other miscellaneous lesions involving bone were noted, however. These included hyperostosis of the bones of the forefoot and hindfoot in one rat, one developmental malformation in the cranium of one rat, and inflammation in one rat. None of the changes was significant.

Special Senses

Eyes. — Two age-related lesions were noted with relative frequency in the eyes of rats from both studies. These

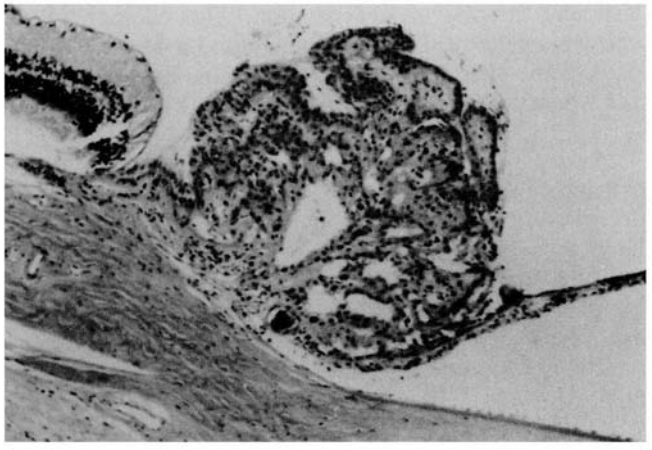

Figure 18. Eye. Enlargement of the ciliary body due to histiocytic infiltration.

changes were cataracts and histiocytic infiltration of the ciliary body. The latter change was present in over half of the rats in the C-SS 18 months of age or older and in all of the rats in the 27-month group. No rats under 18 months of age had the change. In the LS, 24/52 rats in the 13–18-month group, 20/32 rats in the 19–20-month group, 8/14 rats in the 21–22-month group, and 2/2 rats in the 23–24-month group also had histiocytic infiltration in the ciliary body (Figure 18). The cause of this change and the origin of these cells are unknown.

Unilateral or bilateral cataracts were present in the lens of numerous rats, and the incidence increased with age. While a cataract was noted in one 12-month-old rat in the C-SS, most cataracts were diagnosed in rats 21 months of age or older. Nine of ten rats in the 27-month group had cataracts. In the LS, of the 25 rats with cataracts, 17 were in the 19–24-month group and 7 were older than 21 months.

Tympanic bulla. — No primary lesions were noted. One rat was affected by a hematopoietic neoplasm (mononuclear cell leukemia).

External ear. — No lesions were present in the C-SS. In the LS, two rats had neoplasms (carcinomas) of the Zymbal's gland (auditory sebaceous gland). One schwannoma was present in and around the external ear of one rat from the LS. The origin of the tumor was not determined.

Hematopoietic System

Bone marrow. — Changes in the bone marrow were found in only one rat, which had mononuclear cell leukemia.

Lymph nodes. — A large number of incidental lesions were noted at necropsy in various lymph nodes. The most frequent finding was hemorrhage, and the mediastinal lymph nodes were the most frequent site. Far more lymph node lesions were noted in the LS than in the C-SS and were probably related to the disease conditions found in some of the moribund rats.

Miscellaneous. — Mononuclear cell leukemia was noted in multiple organs of one 27-month-old C-SS rat.

Integumentary System

Skin. — Numerous non-neoplastic and neoplastic lesions of the skin were found at necropsy and examined microscopically. Most of the lesions involved the tail and were age-related. None was present in the C-SS in rats less than 21 months of age. Non-neoplastic lesions were primarily inflammatory. Neoplastic lesions included basosquamous tumor, fibroma, fibrosarcoma, keratoacanthoma, lipoma, and squamous papilloma, and all were infrequent.

Discussion

The results of the C-SS and LS produced general agreement concerning the pathological profile of the GRC Wistar rat. It was evident that the major pathology and leading cause of moribundity of this strain was kidney disease, manifested as chronic nephropathy and hydronephrosis. This observation agrees with identified pathology in other rat strains, including Fischer 344 (Coleman et al., 1977; Goodman et al., 1979; Maeda et al., 1985), Sprague-Dawley (Anver et al., 1982) and Wistar rats (Malkowa et al., 1983). The extent to which this pathology in GRC Wistar rats caused other pathological changes, e.g., parathyroid changes, remains unknown. Also unknown was the interaction of diet with disease. Masoro et al. (1989) noted marked influence on the incidence of nephropathy in Fischer 344 rats associated with the amount and type of dietary protein. In addition, dietary restriction over a major portion of this life span markedly reduced the incidence of nephropathy in this strain (Maeda et al., 1985) as has also been observed in the Lobund-Wistar rat (Snyder et al., 1990). Although no pathological assessment was conducted, Goodrick et al. (1982) noted that mortality was heavily reduced in GRC Wistar rats subjected to dietary restriction by every-other-day feeding, but it is likely that this effect was associated with reduced incidence of nephropathy.

In contrast, incidence of neoplasms was relatively low among GRC Wistar rats, compared to other strains (e.g., Goodman et al., 1979). Adenoma of the pars distalis of the pituitary gland was perhaps the only notable neoplasm. This low incidence of neoplasms for this strain can be viewed favorably in comparison to that observed in the widely used Fischer 344 rat, which shows a high incidence of pituitary adenomas (Coleman et al., 1977; Maeda et al., 1985) and mononuclear cell leukemia (Coleman et al., 1977; Losco and Ward, 1984). However the relatively low median life span observed in the GRC of Wistar rats colony may have prevented a greater incidence of neoplasms. Among the longer-lived Lobund-Wistar strain, tumors do not usually appear until about 18 months, with most not found until 30 months of age. However, among the outbred Sprague-Dawley strain, which tends toward great body size and adiposity compared to other strains, a high incidence of tumors is notable at ages more in line with the shorter life spans observed in GRC Wistar rats (Ross and Bras, 1971). Tumor incidence was again very responsive to diet manipulation in this strain.

Instead, several other major pathologies could be used to characterize GRC Wistar rats raised under the environmental conditions imposed. Among them would be chronic cardiomyopathy. Hyperplasia was notable in several sites and organs, including bile duct, parathyroids, and adrenal medulla. It remains to be determined if the hyperplasia represents a disease or a normal aging process similar to the thymic atrophy observed. Other sites more suspicious for disease processes would include the chronic inflammation of the preputial gland, and lymphoid infiltration and granulomatous inflammation of the lungs. These conditions could perhaps be altered by changes made in the pathogen environment, such as the impact of PVM on lung function. Major pathology to sensory organs was restricted to the eyes, where cataracts and histiocytic infiltration of the ciliary body were noted and to the areas where axonal degeneration of the cauda equina was observed. Light exposure as well as other possible environmental factors might be responsible for the incidence of cataracts. Unobserved in this colony to any degree was neurogenic muscular atrophy, observed in Wistar rats but from a colony that was much longer-lived than that reported here (Van Steenis and Kroes, 1971).

Thus, it is possible that manipulation of environmental factors (e.g., diet, SPF status, lighting) could impact on the pattern of pathology noted in this study. In addition, it is likely that such manipulations would impact favorably upon mortality, which in turn might unveil other pathologies emerging at later ages. The median survival age for the GRC Wistar rat has ranged from 20–24 months, which accords with previous reports involving this strain (Goodrick et al., 1982). This estimate agrees with earlier reports for male Wistar rats (Paget and Lemon, 1965), but is markedly less than that reported for male Wistar rats in other colonies (Beauchene et al., 1986; Snyder et al., 1990). However, marked variation in estimates of colony life span is well known (e.g., Schlettwein-Gesell, 1970), which underscores the role of environmental factors related to such estimates. Moreover, the great genetic variation within the outbred Wistar strain could produce this variation in life span estimates, as well as pathology. Indeed, because of the diverse background of this rat and its origin, it is difficult to even consider them a strain (Hansen, 1979).

Strategies for the development of ideal rodent models for gerontological research have been under consideration for nearly two decades (Gibson, 1972; National Research Council, 1981). The major challenge has been how to develop a model in which the investigator can disentangle the effects of aging from the contamination of specific predominant disease. Indeed, this same problem continues to face human research (Fozard et al., 1990). For rodent research the challenge remains to identify strains for which there is ". . . no single outstanding genetic sensitivity to infectious, degenerative, or neoplastic disease" (Grahn, 1972). Now, however, environmental factors, and particularly diet, can also be considered (Weindruch and Masoro, 1991).

For the GRC-Wistar rat, as with any strain used for aging research, it is essential that investigators ensure that age-related data are not altered by the disease state of the animal. Environmental manipulations, such as dietary modification, may be considered to reduce specific pathologies, nephropathy, and neoplasms. Finally, since no truly "ideal" rodent models currently exist, comparative data from several strains are important to determine the general applicability of findings to the mammalian aging processes.

The GRC Wistar Rat Colony consists of approximately 12,000 outbred animals, ranging in age from birth to the near limits of their life expectancy. This colony, representative of the oldest line of rats bred for laboratory use, has provided models for in vivo research to investigators in nearly every section within the NIA intramural research program, as well as to scientists throughout the country. It is the current intent of NIA to continue the maintenance of this colony, because the very large data base that has been developed over the years is a valuable resource. Various experiments to reduce specific pathologies, such as kidney disease, through changes in diet and housing are ongoing or planned.

ACKNOWLEDGMENTS

The authors acknowledge the thoughtful inspiration of the late Dr. Bennett J. Cohen, who was a driving force in the design of this study. Thanks are also due to Peggy Betkey, the late Rita Wolferman, Catherine Scaffidi, and Valerie Robinson for the typing of the manuscript. In addition, the authors would like to thank Larry Wongus for his dedication with the animals throughout the study.

The Animal Resources Section's Animal Care and Use Program is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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Received June 2, 1992
Accepted November 4, 1992