Pathological Changes During Aging in Barrier-Reared Fischer 344 Male Rats¹

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Pathology, microbiology, and selected serum chemistries were evaluated in 144 male Fischer rats from 4 to 33 mo of age. The rats were reared and maintained under barrier conditions, which successfully excluded the introduction of major infectious disease agents throughout the entire study, including *Mycoplasma pulmonis*. A wide variety of pathology was found and tabulated, and many lesions were found to increase in severity and incidence with age. There was a high correlation of renal disease severity with increasing age. Serum total protein and albumin decreased with age, while alpha-1 globulin and cholesterol increased.

THE choice of a laboratory animal for a particular study should be based on the best information available. For example, male F344 rats have a high incidence of testicular interstitial cell tumors (Jacobs & Huseby, 1967). This fact may make these rats valuable for some studies and useless for others. Without baseline information, choosing a strain of animal could waste time and money. Furthermore, lack of information about more subtle changes affecting certain organ systems could influence interpretation of data. Once an animal is selected, it must be free of most infectious diseases and must be maintained under controlled environmental conditions to minimize introduction of life-limiting variables. These influences become especially important when life-span studies are contemplated.

This report details data obtained from specific pathogen free F344 male rats. Parameters for this study included morphological alterations of major organs and measurement of selected serum chemicals. The effort was not to relate findings to the aging process *per se*, but rather to obtain data that may be of value to investigators using F344 rats. In order to obtain meaningful information, the rats had to be free of significant infectious disease. Thus, serological and microbiological monitoring was a

major aspect of the study. The study also provided information on the feasibility of maintaining rats free of major intercurrent infection throughout their life-span in a commercial breeding environment and of shipping aged rats to investigators without significant mortality or stress.

MATERIALS AND METHODS

Rats. — Fischer 344 rats were obtained by hysterectomy and were raised axenically in flexible film isolators. Rats from this nucleus stock were inoculated orally with a mixed broth bacterial culture containing a modification of the Schaedler formula (Schaedler, Dubos, & Costello, 1965). Breeding stock derived from the nucleus colony were maintained under barrier husbandry conditions in a standard commercial production building at the Charles River Breeding Laboratories, Wilmington, MA.

At the time of birth, large litters were reduced to 8 young. When weaned, the sexes were separated, and male rats were maintained at 5 per cage until 6 mo of age then at 3 per cage without additions or recombinations thereafter. The rats were housed in shoebox type cages, 13" x 12" by 7" with heat-sterilized hardwood bedding. They were fed a commercial diet *ad libitum* (Charles River 4RF Rat-Mouse Formula), which contained a minimum of 26% protein, 5% fat, and a maximum of 5.0% fiber. The diet was pasteurized (225F for 15 min) but was not sterilized.

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The first group of 15 male rats was established in April, 1970. Groups of 15 rats were added to the colony each succeeding month for 6 mo (total 90), then groups of 30 rats were added monthly (total 360). Rats from these cohorts were utilized for the age-related pathology characterization.

Pathology. — One hundred forty-four rats were necropsied and evaluated histopathologically; 9 of these rats were found dead, and 28 were moribund when euthanized. Early in this study, 29 rats, classified as "unhealthy" due to clinically detectable lesions or major weight loss, were euthanized and necropsied. This procedure was discontinued when it was realized that culling of unhealthy animals from the colony could alter life-span calculations. Subsequent rats were killed only if moribund, or according to a predetermined schedule using a table of random numbers.

Rats were anesthetized with ether, chloroform, or CO₂ and immersed in a disinfectant solution. The abdomen was open aseptically, and rats were killed by exsanguination via the posterior vena cava.

Samples of brain, lumbar spinal cord, pituitary, eyes, trachea, thyroid, lung, heart, liver, kidneys, urinary bladder, spleen, pancreas, adrenal glands, testes, seminal vesicles, prostate, triceps brachii and quadriceps femoris muscles, elbow and knee joints, sternum and vertebrae, and all additional gross lesions were routinely collected and examined histologically. Tissues were fixed in 10% neutral buffered formalin and occasionally in Bouin's fixative. The gastrointestinal tract and lungs were fixed by perfusion. Tissues were embedded in paraffin, sectioned at 6 μ m and stained with hematoxylin and eosin. Selected tissues were sectioned at 3 μ m and stained by: periodic acid Schiff's, Perl's, Masson's trichrome, phosphotungstic acid hematoxylin, Laidlaw's connective tissue, elastica VonGiesson, von Kossa, Giemsa, Voght's, Bodian, Kluver's and Masson's methenamine silver stains.

Clinical chemistry. — Serum cholesterol, creatinine, urea nitrogen, sodium, potassium, total protein, albumin, and levels of globulins were determined by established methods: sodium and potassium by flame photometry, total protein by Biuret Method, and protein fractionation by electrophoresis, using a cellulose acetate membrane and quantitation by densitometric measurement. Cholesterol and creatinine were measured colorimetrically

(Van Pilsum, Martin, Kito, & Hess, 1956; Zlatkis, Zak, & Boyle, 1953).

Microbiology. — Saline aspirates of nasopharynx and tympanic bullae were cultured for *Mycoplasma pulmonis* and *Pseudomonas aeruginosa*. The cecum was cultured for *Salmonella* and *P. aeruginosa*, and contents were examined for intestinal helminths. Gross lesions were cultured at the discretion of the pathologist.

Serology. — Coded samples of frozen serum were shipped to Microbiological Associates⁷ for detection of hemagglutination inhibition (HAI) antibody to pneumonia virus of mice (PVM), Theiler's encephalomyelitis virus (GDVII), Sendai virus, minute virus of mice (MVM), Kilham rat virus (KRV), Toolan H-1 virus, and complement fixing (CF) antibody to murine adenovirus, murine hepatitis virus (MHV), lymphocytic choriomeningitis virus (LCM) and rat coronavirus (RCV).

Statistical analysis. — Inter-relationships were sought between age and nephropathy severity index, age and serum chemistry values, nephropathy and serum chemistry values, and between selected serum chemical parameters, using correlation coefficients. Coefficients greater than 0.30 were considered significantly correlated ($p = 0.99$), based on the sample size. When a correlation between 2 parameters was found, the linear regression formula was calculated. Calculations and determination of probability levels were performed by established methods (Steel & Torrie, 1960).

RESULTS

Pathology. — A wide variety of lesions occurred. The type and incidence of lesions in various organs are tabulated in Tables 1 through 19 and are discussed below. All rats had one or more lesions, and a majority of dead or moribund rats had some form of neoplasia or advanced renal disease. Rats selected as "unhealthy" also had some form of neoplasia, and 2 had posterior paresis of undetermined origin.

Neoplasms: A few tumors were seen in rats less than 18 mo of age, but most occurred after that point (Table 1). The most common tumor types were testicular interstitial cell tumors,

⁷Microbiological Associates, Bethesda, MD.

pituitary chromophobe adenomas, and a form of mononuclear cell leukemia. The incidence of other tumors was low. A total of 103 rats had some form of neoplasia. Thirty-five of the affected rats had 2, 30 rats had 3, 6 rats had 4, 2 rats had 5, and 1 rat had 6 types of neoplasia at one time. The tumors are discussed under the individual organ categories below.

Although leukemia is generally rare in rats, there is an unclassified mononuclear cell leukemia that has reached an incidence of up to 25% in the Wistar-Furth (W-Fu) and Fischer rat strains (Davey & Moloney, 1970; Jacobs & Huseby, 1967; Moloney, Boschetti, & King, 1968, 1970). Twenty-three rats in this study

(16%) had leukemia of this type. The highest incidence was 29.8% in the 24- to 30-mo age group. Fourteen rats were clinically ill prior to necropsy and the remainder were asymptomatic. Hemograms on 9 of these rats revealed a total leukocyte count range of 68,400 to 323,000 cells/ μ l (average 143,190). The cells were morphologically identical to those described by others (Moloney et al., 1970). Minimal organ changes, related to the leukemia, were present. Twenty rats had spleens ranging from 4 to 20 times normal size. Splenomegaly was due to sequestration of erythrocytes and neoplastic mononuclear cells in the sinuses of the red pulp. There was effacement of the white pulp.

Table 1. Neoplasms.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total Cases	Total Examined	% Over-all Incidence
Testicular interstitial cell tumor	—	—	4(21.1) ^a 3,-,1	28(80.0) 7,14,7	41(97.6) 22,7,2	15(100) 9,3,3	88 41,24,23	133	(66.2)
Mononuclear cell leukemia	—	—	1(5.0) -, -, 1	7(17.5) 1,2,4	14(29.8) 5,-,9	1(6.7) -, -, 1	23 6,2,15	144	(16.0)
Pituitary chromophobe adenoma	—	1(7.7) 1,-,-	—	10(25.6) 1,4,5	8(17.0) 4,1,3	2(13.3) 2,-,-	21	143	(14.7)
Preputial gland adenoma	—	1(7.7) 1,-,-	1(5.0) -, 1,-	3(8.1) -,3,-	4(8.7) 1,1,2	1(6.7) 1,-,-	10	140	(7.1)
Fibroma, subcutaneous & dermal	—	—	—	7(18.9) -,5,2	1(2.2) -, -, 1	2(13.3) 1,-,1	10	140	(7.1)
Pancreatic islet cell adenoma	—	—	—	1(2.5) -, -, 1	3(6.4) -, -, 3	5(33.3) 4,1,-	9 4,1,4	144	(6.2)
Pheochromocytoma	—	—	—	2(5.0) -, -, 2	2(4.3) -, -, 2	1(6.7) 1,-,-	5 1,-,4	144	(3.5)
Lipoma	—	—	1(5.0) -, -, 1	2(5.0) -,2,-	1(2.1) 1,-,-	1(6.7) -,1,-	5 1,3,1	144	(3.5)
Auditory sebaceous tumors	—	—	—	2(5.4) 1,1,-	—	2 ^b (13.3) 2,-,-	4 3,1,-	140	(2.9)
Mesothelioma, testicular	—	—	—	2(5.7) -, -, 2	—	1(6.7) -, -, 1	3 -, -, 3	133	(2.3)
Basal cell tumors	—	—	1(5.0) 1,-,-	2(5.4) 2,-,-	—	—	3 3,-,-	140	(2.1)
Thyroid medullary carcinoma	—	—	—	—	2(4.3) -, -, 2	1(6.7) 1,-,-	3 1,-,2	144	(2.1)
Mammary adenofibroma	—	—	—	—	2(4.3) 1,-,1	1(6.7) 1,-,-	3 2,-,1	144	(2.1)
Adrenocortical adenoma	—	—	—	—	2(4.3) -,1,1	—	2 -,1,1	144	(1.4)
Cutaneous squamous papilloma	—	—	—	—	1(2.2) -, -, 1	1(6.7) 1,-,-	2 1,-,1	140	(1.4)
Generalized lymphosarcoma	—	—	—	—	1(2.1) -, -, 1	1(6.7) -, -, 1	2 -, -, 2	144	(1.4)
Urinary bladder transitional cell papilloma	—	—	—	1(2.5) -,1,-	—	—	1 -,1,-	144	(0.7)
Hepatocellular carcinoma	—	—	—	1(2.5) -, -, 1	—	—	1 -, -, 1	144	(0.7)
Thyroid adenoma	—	—	—	—	1(2.1) -, -, 1	—	1 -, -, 1	144	(0.7)
Angioma, subcutaneous	—	—	—	—	1(2.1) -,1,-	—	1 -,1,-	140	(0.7)
Rhabdomyosarcoma	—	—	—	1(2.5) 1,-,-	—	—	1 1,-,-	143	(0.7)
Generalized reticulum cell sarcoma	—	—	—	—	1(2.1) 1,-,-	—	1 1,-,-	144	(0.7)

^aThe numbers A (B) indicate: A = # of cases within the age group
C, D, E B = % incidence within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

refer to appropriate organ system table for group size and over-all incidence within individual age group.

^b1 with transformation to squamous cell carcinoma and metastases to lung.

by tumor growth in only 3 of these rats. Infiltration of leukemic cells occurred irregularly in other organs: lung, 4; liver, 8; kidney, 1; adrenal gland, 7; pancreas, 1; skeletal muscle, 1; lymph node, 2; and bone marrow, 9. Seven of the 23 rats had no evidence of organ infil-

tration. This pattern of variable organ involvement was similar to that described by others except the incidence of hepatic and pulmonary involvement in this study was much lower (Davey & Moloney, 1970; Moloney et al., 1970).

Table 2. Respiratory System Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Peribronchial lymphocytic aggregates	2(22.2) 2,-,-	4(30.8) 4,-,-	12(60.0) 11,1,-	24(60.0) 5,12,7	26(55.3) 14,4,8	5(33.3) 4,-,1	73(50.7) 40,17,16
Perivascular lymphocytic infiltrates	5(55.6) 5,-,-	6(46.1) 6,-,-	3(15.0) 3,-,-	4(10.0) -,2,2	2(4.2) 2,-,-	2(13.3) 2,-,-	22(15.3) 18,2,2
Focal chronic interstitial pneumonia	—	1(7.7) 1,-,-	4(20.0) 3,-,1	4(10.0) -,3,1	4(8.5) 3,-,1	—	13(9.0) 7,3,3
Focal atypical pneumonia (adenomatosis)	—	—	—	—	5(10.6) 3,1,1	1(6.7) 1,-,1	6(4.2) 4,1,1
Mononuclear cell leukemic infiltrates	—	—	1(5.0) -,-,1	2(5.0) -,-,2	1(2.1) -,-,1	—	4(2.8) -,-,4
Medial calcification pulmonary artery	—	—	—	1(2.5) -,-,1	2(4.2) -,1,1	—	3(2.1) -,1,2
Medial hypertrophy pulmonary artery	1(11.1) 1,-,-	—	—	—	2(4.2) 1,-,1	—	3(2.1) 2,-,1
Alveolar phagocytosis	—	—	—	—	—	2(13.3) 1,-,1	2(1.4) 1,-,1
Focal interstitial fibrosis	—	1(7.7) 1,-,-	—	—	—	—	1(0.7) 1,-,-
Emphysema	—	1(7.7) 1,-,-	—	—	—	—	1(0.7) 1,-,-
Metastatic squamous cell carcinoma	—	—	—	—	—	1(6.7) 1,-,-	1(0.7) 1,-,-
No changes	3(33.3) 3,-,-	3(23.1) 3,-,-	4(20.0) 4,-,-	7(17.5) 3,3,1	16(34.0) 7,3,6	6(40.0) 3,2,1	39(27.1) 23,8,8

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E
B = % of cases within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 3. Cardiac Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Myocardial fibrosis	—	6(46.1) 6,-,-	7(35.0) 7,-,-	30(75.0) 8,12,10	36(76.6) 15,5,16	13(86.7) 8,2,3	92(63.9) 44,19,29
Focal chronic myocarditis	8(88.9) 8,-,-	11(84.6) 11,-,-	10(50.0) 10,-,-	11(27.5) 1,6,4	2(4.2) 2,-,-	3(20.0) 2,-,1	45(31.2) 34,6,5
Focal myocardial degeneration	2(22.2) 2,-,-	5(38.5) 5,-,-	11(55.0) 11,-,-	10(25.0) 3,6,1	6(12.8) 4,1,1	3(20.0) 1,1,1	37(25.7) 26,8,3
Focal hemosiderin deposits	—	—	4(20.0) 4,-,-	1(2.5) -,1,-	—	—	5(3.5) 4,1,-
Chronic valvular endocarditis	—	—	1(5.0) 1,-,-	1(2.5) 1,-,-	1(2.1) -,-,1	—	3(2.1) 2,-,1
Coronary arteritis with sclerosis	—	1(7.7) 1,-,-	1(5.0) 1,-,-	—	—	—	2(1.4) 2,-,-
Medial hypertrophy coronary artery	—	—	—	1(2.5) 1,-,-	—	—	1(0.7) 1,-,-
Medial lipid coronary artery	—	—	—	—	1(2.1) -,-,1	—	1(0.7) -,-,1
No changes	1(11.1) 1,-,-	2(15.4) 2,-,-	5(25.0) 2,1,2	8(20.0) 1,4,3	9(19.1) 6,2,1	2(13.3) 1,1,-	27(18.7) 13,8,6

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C,D,E
B = % of cases within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Respiratory System: The most frequent finding was small lymphocytic aggregates in or just beyond the walls of major bronchi (Table 2). The incidence of these increased with age until 12 mo, then remained steady until 30 mo, and then declined. Mild perivascular lymphocytic infiltrates were found in most rats less than 6 mo of age, and the incidence decreased steadily with age. There were several cases of mild focal chronic interstitial pneumonia, characterized by small foci of alveolar septal fibrosis with sparse infiltrates of lymphocytes and macrophages. These were generally adjacent to peribronchial lymphocytic aggregates. One rat had focal interstitial fibrosis without leukocytic infiltrates.

The most severe pulmonary lesion was a mild focal atypical hyperplasia (Innes, Garner, & Stookey, 1967) which occurred in a few rats over 24 mo of age. Alveoli were lined by cuboidal to columnar epithelial cells, with variable amounts of interstitial fibrosis. Three rats had

medial hypertrophy, and 3 had medial calcification of the pulmonary artery, both of which are considered to occur frequently in rats (Wilens & Sproul, 1938). A few rats in the oldest age group had focal intraalveolar collections of foamy macrophages primarily in subpleural regions. This has been considered a normal finding in rats (Beaver, Ashburn, & McDaniel, 1963). None of the lungs had lesions suggestive of chronic respiratory disease, a disease associated with *Mycoplasma pulmonis* (Jersey, Whitehair, & Carter, 1973).

No primary pulmonary neoplasms were detected. Four rats with a mononuclear cell leukemia had mild alveolar septal infiltrates, and 1 rat had pulmonary metastases of a squamous cell carcinoma arising in the ear canal.

Other common findings, not tabulated in this study, were dilatation and atrophy of tracheal glands and mineralization of tracheal cartilage.

Cardiovascular System: Cardiac lesions were common and appeared to be influenced

Table 4. Urinary System Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Chronic Nephropathy							
Grade 1	8(88.9) 8,-,-	6(46.2) 6,-,-	4(20.0) 3,-,1	1(2.5) -,1,-	1(2.1) -,,-1	—	20(13.2) 17,1,2
Grade 2	—	6(46.2) 6,-,-	11(55.0) 10,-,1	6(15.0) 1,3,2	—	—	23(16.0) 17,3,3
Grade 3	—	—	5(25.0) 3,1,1	14(35.0) 3,6,5	5(10.6) 2,1,2	2(13.3) 1,1,-	26(18.1) 9,9,8
Grade 4	—	1(7.7) 1,-,-	—	18(45.0) 4,8,6	31(66.0) 15,7,9	6(40.0) 4,1,1	57(39.6) 24,16,16
End stage	—	—	—	1(2.5) -,,-1	10(21.3) 5,-,5	7(46.7) 4,1,2	18(12.5) 9,1,8
Total	8(88.9) 8,-,-	13(100) 13,-,-	20(100) 16,1,3	40(100) 8,18,14	47(100) 22,8,17	15(100) 9,3,3	143(99.3) 76,30,37
Tubular lipochrome pigment	3(33.3) 3,-,-	8(61.5) 8,-,-	17(85.0) 14,1,2	40(100) 8,18,14	47(100) 22,8,17	15(100) 9,3,3	130(90.3) 64,30,36
Chronic interstitial nephritis	—	4(30.8) 4,-,-	13(65.0) 12,1,-	25(62.5) 3,13,9	33(70.2) 16,7,10	11(73.3) 7,3,1	86(59.7) 42,24,20
Healed renal infarct	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Metastatic pheochromocytoma	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Renal reticulum cell sarcoma	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Renal lymphosarcoma	—	—	—	—	1(2.1) -,,-1	—	1(0.7) -,,-1
Renal mononuclear cell leukemic infiltration	—	—	—	—	1(2.1) -,,-1	—	1(0.7) -,,-1
Acute pyelonephritis with cystitis	—	—	—	1(2.5) -,,-1	—	—	1(0.7) -,,-1
Suppurative cystitis	—	—	—	—	—	1(6.7) 1,-,-	1(0.7) 1,-,-
Bladder transitional cell papilloma	—	—	—	1(2.5) -,,-1	—	—	1(0.7) -,,-1

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C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

by age (Table 3). Mild to moderate focal chronic interstitial myocarditis occurred frequently in rats less than 6 mo old and then decreased in incidence with increasing age. It was characterized by focal aggregates of large mononuclear leukocytes and scattered phagocytic cells which separated myocardial fibers. Myocardial degeneration occurred with or without inflammation and was characterized by focal regions of shrunken or vacuolated myocardial fibers. These two lesions were not always coincidental, but one or both appeared to be the predecessor of interstitial fibrosis, which was first seen at 6 to 12 mo, and increased in incidence and degree with age. This lesion has been well reviewed by others (Berg, 1967; Fairweather, 1967). The fibrosis appears to be closely related to early degenerative and inflammatory changes, rather than based on a vascular origin. Only a few vascular or valvular lesions were found, which seemed to have no correlation to age. None of the lesions noted were considered of sufficient severity to impair cardiac function.

Vascular lesions, which occurred rarely, are discussed under the organ system involved.

Urinary System: All but one of 144 rats examined had some degree of renal pathology (Table 4) and that rat was less than 6 mo old. The lesion common to all rats was a complex of chronic changes that have been given a variety of names such as nephritis, nephrosis, glomerulosclerosis, glomerular hyalinosis, nephropathy, and chronic progressive nephrosis (Andrew & Pruett, 1957; Berg, 1965; Blatherwick & Medlar, 1937; Couser & Stilman, 1975; Durand, Fisher, & Adams, 1964; Elema & Arends, 1975; Elema, Koudstaal, Lamberts, & Arends, 1971; Foley, Jones, Osborn, & Kimeldorf, 1964; Gray, Weaver, & Purmalis, 1974; Lalich & Allen, 1971; Lalich, Faith, & Harding, 1970; Medlar & Blatherwick, 1937; Saxton & Kimball, 1941; Simms & Berg, 1957). The term "chronic nephropathy" was chosen in this study as the best descriptive term for this disease, since the lesion was chronic, was more complex than the aforementioned specific terms imply, and involved the entire nephron. The pathogenesis, histopathology, and ultrastructural pathology of the nephropathy have recently been well described (Couser & Stilman, 1975; Elema & Arends, 1975; Gray et al., 1974).

Chronic nephropathy was graded 1 to 4, with an additional, end stage, category for severely affected kidneys. Grade 1 kidneys had thick-

ened glomerular capillary basement membranes and slight mesangial thickening in some glomeruli. A few cortical tubules were shrunken, had thickened, wrinkled basement membranes, and were lined by enlarged cells containing basophilic cytoplasm and large nuclei. In addition, Grade 2 kidneys had scattered dilated tubules lined by atrophic epithelium with occasional hyalin casts, particularly at the corticomedullary junction. Protein casts became prominent in Grade 3 kidneys, and appeared within cortical, medullary, and papillary tubules. The glomerular lesion was more pronounced with atrophy of capillary tufts, sclerosis, thickening of Bowman's capsule, and tubular basement membranes. These findings were pronounced in Grade 4 kidneys, which commonly had adhesions of glomerular tufts to Bowman's capsule and both enlarged nuclei in the tunica media and proliferation of adventitial connective tissue in afferent arterioles of the severely affected glomeruli. End-stage disease had no normal parenchyma remaining, with widespread glomerular sclerosis, marked tubular dilation, atrophy and hyalin cast formation, and interstitial fibrosis.

Cytoplasmic tubular epithelial lipochrome pigment increased with age and first appeared at 150 days. In severe cases, it was accompanied by hemosiderin granules. Scattered aggregations of interstitial mononuclear leukocytes (chronic interstitial nephritis) were frequently found throughout the cortex but could not be correlated with the severity of nephropathic change.

Fig. 1. Significant relationship of increasing age to increasing severity of chronic nephropathy (see text for index criteria) in all rats submitted for necropsy ($p = 0.99$).

Table 5. Splenic Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Chronic passive congestion	—	—	—	7(17.5) -,1,6	22(46.8) 7,2,13	9(60.0) 6,1,2	38(26.4) 13,4,21
Healed infarct	—	—	—	—	3(6.4) -,1,2	3(20.0) 2,1,-	6(4.2) 2,2,2
Reticuloendothelial hyperplasia	—	—	1(5.0) 1,-,-	1(2.5) -,-,1	3(6.4) -,-,3	1(6.7) -,-,1	6(4.2) 1,-,5
Extramedullary hematopoiesis	—	—	—	3(7.5) 2,1,-	2(4.3) 1,1,-	—	5(3.5) 3,2,-
New infarct	—	—	—	1(2.5) -,1,-	2(4.3) -,-,2	—	3(2.1) -,1,2
Mononuclear cell leukemic infiltrate (white pulp)	—	—	—	1(2.5) -,-,1	1(2.1) -,-,1	1(6.7) 1,-,-	3(2.1) 1,-,2
Lymphoid depletion	—	—	—	1(2.5) -,-,1	—	—	1(0.7) -,-,1
Reticulum cell sarcoma	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Lymphosarcoma	—	—	—	—	1(2.1) -,-,1	—	1(0.7) -,-,1
No changes	9(100) 9,-,-	13(100) 13,-,-	19(95.0) 15,1,3	29(72.5) 5,16,8	23(48.9) 15,4,4	6(40.0) 3,2,1	99(68.7) 60,23,16

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 6. Adrenal Gland Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
<i>Cortex:</i>							
Fatty change	—	2(15.4) 2,-,-	2(10.0) 1,-,1	4(10.0) 1,-,3	3(6.4) 2,-,1	4(26.7) 2,1,1	15(10.4) 8,1,6
Mononuclear cell leukemic infiltration	—	—	—	1(2.5) -,1,-	3(6.4) 1,1,1	3(20.0) 2,-,1	7(4.9) 3,2,2
Telangiectasis	—	—	1(5.0) 1,-,-	1(2.5) -,-,1	2(4.3) -,-,2	2(13.3) 1,-,1	6(4.2) 2,-,4
Nodular hyperplasia	—	—	—	—	1(2.1) -,-,1	1(6.7) -,1,-	2(1.4) -,1,1
Adenoma	—	—	—	—	2(4.3) -,1,1	—	2(1.4) -,1,1
Diffuse hypertrophy	—	—	—	—	1(2.1) -,-,1	—	1(0.7) -,-,1
Focal suppurative pericapsular inflammation	—	—	—	1(2.5) -,-,1	—	—	1(0.7) -,-,1
Periarteritis, capsular artery	—	—	—	1(2.5) -,-,1	—	—	1(0.7) -,-,1
<i>Medulla:</i>							
Pheochromocytoma	—	—	—	2(5.0) -,-,2	2(4.3) -,-,2	1(6.7) 1,-,-	5(3.5) 1,-,4
Hyperplasia	—	—	—	1(2.5) -,-,1	1(2.1) -,-,1	—	2(1.4) -,-,2
Thrombosis	—	—	—	1(2.5) -,-,1	1(2.1) -,-,1	—	2(1.4) -,-,2
Mononuclear cell leukemic infiltration	—	—	—	—	1(2.1) -,-,1	—	1(0.7) -,-,1
No changes	9(100) 9,-,-	10(76.9) 10,-,-	18(90.0) 14,1,3	32(80.0) 7,18,7	31(66.0) 19,4,8	8(53.3) 5,1,2	108(75.0) 64,24,20

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

The incidence and severity of chronic nephropathic changes are presented in Table 4 and plotted against age in Fig. 1. There was a highly significant correlation between aging and severity of the renal disease (correlation coefficient = +0.80, $n = 144$ rats).

Other renal lesions occurred sporadically. One rat had a large infarct of unknown cause. Tumor metastases occurred in several rats and included: reticulum cell sarcoma, lymphosarcoma, and mononuclear cell leukemic infiltration. A pheochromocytoma had invaded the perirenal tissue of 1 rat.

Urinary bladder lesions consisted of two cases of suppurative cystitis and one of transitional cell papilloma. Cystitis occurred concurrently with pyelonephritis in a 600-day-old paralyzed, moribund rat, apparently secondary to neurogenic atony.

Splenic Lesions: Lesions were absent in rats less than 18 mo old, except for reticuloendothelial hyperplasia in 1 rat in the 12- to 18-mo group (Table 5). Chronic passive congestion was evident in spleens of rats with severe bile duct hyperplasia. Circulatory embarrassment, related to hepatic portal fibrosis, was thought to be the cause of the splenic congestion. In 20

rats with mononuclear cell leukemia, splenic congestion was exacerbated by infiltration of the hepatic sinusoids by leukemic cells, in addition to primary leukemic involvement of the spleen. Marked leukemia-associated splenomegaly has been noted by others (Davey & Moloney, 1970; Jacobs & Huseby, 1967; Moloney et al., 1969, 1970). Infiltration of white pulp occurred in only three cases. In 5 rats with passive congestion, there was reticuloendothelial hyperplasia. The spleens of 6 rats in the two oldest age groups had recent or old infarcts, possibly related to chronic passive congestion. Excessive extramedullary hematopoiesis occurred secondary to anemia in 5 rats.

Endocrine System: Adrenal cortical changes were not evident before 6 mo. In rats with neoplasms, especially chromophobe adenomas of the pituitary, there was diffuse or focal fatty change in the inner cortex (Table 6). Cortical adenomas were found in 2 rats of the 24- to 30-mo group. Two rats in the older age group also had hyperplastic nodules in the zona fasciculata, and 1 rat had diffuse cortical hypertrophy. Telangiectasis of cortical vessels was present in 6 rats more than 12 mo old. Two rats

Table 7. Pancreatic Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Islet cell adenoma	—	—	—	1(2.5) -,1	3(6.4) -,3	5(33.3) 4,1,-	9(6.2) 4,1,4
Lobular atrophy	—	—	—	2(5.0) -,2,-	3(6.4) -,3	1(6.7) 1,-,-	6(4.2) 1,2,3
Acinar ectasia	—	1(7.7) 1,-,-	—	—	—	2(13.3) 2,-,-	3(2.1) 3,-,-
Focal healed pancreatitis	—	—	1(5.0) 1,-,-	1(2.5) -,1,-	—	1(6.7) 1,-,-	3(2.1) 2,1,-
Ductal ectasia	—	1(7.7) 1,-,-	—	—	—	1(6.7) 1,-,-	2(1.4) 2,-,-
Resolved hemorrhage	—	1(7.7) 1,-,-	—	—	1(2.1) 1,-,-	—	2(1.4) 2,-,-
Periarteritis nodosa of Pancreaticoduodenal art.	—	—	—	1(2.5) 1,-,-	—	1(6.7) 1,-,-	2(1.4) 2,-,-
α cell degeneration	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Thrombosis	—	—	—	—	1(2.1) -,1	—	1(0.7) -,1
Mononuclear cell leukemic infiltration	—	—	—	—	1(2.1) -,1	—	1(0.7) -,1
Lymphosarcoma	—	—	—	—	1(2.1) -,1	—	1(0.7) -,1
No changes	9(100) 9,-,-	12(92.3) 12,-,-	19(95.0) 15,1,3	36(90.0) 7,16,13	39(83.0) 20,7,12	8(53.3) 3,2,3	123(85.4) 66,26,31

^aThe numbers A (B) indicate: A = # of cases within the age group
C.D.E., B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

had inflammatory lesions. One had a focal pericapsular suppurative process, and 1 had periarthritis of a capsular artery.

Adrenal medullary changes did not appear in rats less than 18 mo old. Thereafter, only a few lesions were found. Five rats had pheochromocytomas, one of which had spread to perirenal tissues, and another of which occurred concurrently with a thyroid medullary carcinoma. Two rats had thrombosed adrenal medullary vessels, and in 1 rat, thrombosis was associated with hyperplasia of medullary cells. One case of focal medullary hyperplasia occurred without a demonstrable cause. Focal mononuclear cell leukemic infiltrates occurred in the cortex of 7 rats and medulla of 1 rat.

Pancreatic islet lesions occurred in low incidence (Table 7) and are discussed under digestive system.

Hypophyseal lesions were restricted to the adenohypophysis (Table 8). Chromophobe adenomas were commonly diagnosed after 18 mo but one case was found in the 6- to 12-mo group. The tumors were of papillary, sinusoidal, diffuse and mixed types (Russfield, 1968). Compression atrophy of the overlying brain occurred in 6 rats. Nodular hyperplasia was seen in 1 rat. Chromophobe adenomas are common in some strains of rats (Ito, Moy, Kaunitz, Kortwright, Clarke, Furth, & Meites, 1972). Sinusoidal dilatation occurred in 5 rats, and 1 rat had multiple colloid cysts lined by low cuboidal epithelium, and which contained basophilic, hyalin material.

Only a few changes were found in the thyroid and parathyroid glands (Table 9). One rat had diffuse hypertrophy, 1 had an adenoma, and 3 had medullary carcinomas of the thyroid. A higher incidence of thyroid tumors in Fischer

Table 8. Adenohypophyseal Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	39	47	15	143
	9,-,-	13,-,-	16,1,3	8,18,13	22,8,17	9,3,3	77,30,36
Chromophobe adenoma	—	1(7.7) 1,-,-	—	10(25.6) 1,4,5	8(17.0) 4,1,3	2(13.3) 2,-,-	21(14.7) 8,5,8
Sinusoidal dilatation	—	1(7.7) 1,-,-	—	1(2.6) -,1,-	2(4.3) 1,1,-	1(6.7) 1,-,-	5(3.5) 3,2,-
Colloid cyst	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Nodular hyperplasia	—	—	—	1(2.6) -,1,-	—	—	1(0.7) -,1,-
No changes	9(100) 9,-,-	11(84.6) 11,-,-	20(100) 16,1,3	27(69.2) 7,12,8	36(76.6) 16,6,14	12(80.0) 6,3,3	115(80.4) 65,22,28

^aThe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 9. Thyroid and Parathyroid Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,14	9,3,3	77,30,37
Medullary thyroid carcinoma	—	—	—	—	2(4.3) -,2	1(6.7) 1,-,-	3(2.1) 1,-,2
Parathyroid hyperplasia	—	—	—	—	—	2(13.3) 2,-,-	2(1.4) 2,-,-
Thyroid adenoma (α)	—	—	—	—	1(2.1) -,1	—	1(0.7) -,1
Diffuse thyroid hypertrophy	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
No changes	9(100) 9,-,-	13(100) 13,-,-	20(100) 16,1,3	40(100) 8,18,14	43(91.5) 21,8,14	12(80.0) 6,3,3	137(95.1) 73,30,34

^aThe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

and Sprague-Dawley rats has been reported (Jacobs & Huseby, 1967; Thompson, Huseby, Fox, Davis, & Hunt, 1961), but unless rats are fed low iodine diets, thyroid tumors are otherwise considered rare (Axelrad & Leblond, 1955; Isler, Leblond, & Axelrad, 1958). The adenoma seen in this study was of the gamma type (Axelrad & Leblond, 1955; Isler et al., 1958). Two rats over 30 mo old had parathyroid hyperplasia. One had end stage, and the other had Grade 3 nephropathy. Two cases of fibrous osteodystrophy were seen but neither were in rats with demonstrable parathyroid hyperplasia.

Digestive System: Hepatic lesions were common (Table 10). The most frequent lesion was bile duct hyperplasia, which increased in incidence and severity with age. This lesion has not previously been described, which suggests an environmental influence was present in this study. The affected livers were brown, firm,

and rough-surfaced. Beginning at about 6 mo, increased numbers of bile ducts were seen in some portal tracts. The numbers of ducts per portal area and the number of portal areas affected increased with time until the incidence in 18-mo-old rats was greater than 98%. Basement membranes of bile ducts became thickened and sclerosed. Bile ductal epithelium underwent hyperplasia and squamoid metaplasia. Eventually, these changes led to obliteration of portal structures, including blood vessels.

The portal lesion was considered to be an antecedent of the nodular regenerative changes seen in older rats. The latter were first seen in a 720-day-old rat and were accompanied by a hepatocellular carcinoma. Nine other rats had regenerative nodules without evidence of neoplastic transformation.

Other hepatic lesions were generally mild and sporadic and included mild focal or diffuse

Table 10. Hepatic Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Bile duct hyperplasia without sclerosis	—	3(23.1) 3,-,-	2(10.0) 2,-,-	—	1(2.1) 1,-,-	—	6(4.2) 6,-,-
with sclerosis	—	5(38.5) 5,-,-	15(75.0) 12,1,2	39(97.5) 7,18,14	46(97.9) 21,8,17	15(100) 9,3,3	120(83.3) 54,30,36
Focal chronic hepatitis	5(55.5) 5,-,-	5(38.5) 5,-,-	13(65.0) 13,-,-	12(30.0) 5,5,2	10(21.3) 7,-,3	7(46.7) 5,2,-	52(36.1) 45,7,-
Cystic sinusoidal dilatation	—	—	2(10.0) 2,-,-	4(10.0) 1,2,1	7(14.9) 5,1,1	4(26.7) 3,-,1	17(11.8) 11,3,3
Focal necrosis	—	2(15.4) 2,-,-	1(5.0) 1,-,-	2(5.0) 1,-,1	6(12.8) 2,-,4	2(13.3) 1,1,-	13(9.0) 7,1,5
Focal fatty change	—	4(30.8) 4,-,-	1(5.0) 1,-,-	3(7.5) -,1,2	3(6.4) 3,-,-	2(13.3) 1,-,1	13(9.0) 9,1,3
Nodular regeneration	—	—	—	1(2.5) -,1	8(17.0) 4,3,1	1(6.7) 1,-,-	10(6.9) 5,3,2
Hydropic change	—	—	1(5.0) -,1	—	5(10.6) 2,-,3	3(20.0) 1,2,-	9(6.2) 3,2,4
Mononuclear cell leukemic infiltration	—	—	—	3(7.5) -,1,2	4(8.5) 1,-,3	1(6.7) 1,-,-	8(5.6) 2,1,5
Diffuse fatty change	—	—	6(30.0) 6,-,-	—	1(2.1) -,1,-	—	7(4.9) 6,1,-
Hepatocellular atrophy	—	—	—	3(7.5) 2,1,-	2(4.3) -,2,-	—	5(3.5) 2,3,-
Lymphosarcoma	—	—	—	—	1(2.1) -,1	1(6.7) -,1	2(1.4) -,2
Diaphragmatic hernia	—	—	—	1(2.5) -,1,-	—	—	1(0.7) -,1,-
Reticulum cell sarcoma	—	—	—	—	1(2.1) 1,-,-	—	1(0.7) 1,-,-
Hepatocellular carcinoma	—	—	—	1(2.5) -,1	—	—	1(0.7) -,1
No changes	4(44.4) 4,-,-	2(15.4) 2,-,-	2(10.0) 1,-,1	1(2.5) 1,-,-	—	—	9(6.2) 8,-,1

^aThe numbers A (B) indicate: A = # of cases within the age group
 C,D,E B = % of cases within the age group
 C = # of randomly selected rats with lesion
 D = # of "unhealthy" rats with lesion
 E = # of dead or moribund rats with lesion

fatty change, hydropic degeneration, and hepatocellular atrophy. The incidence of acute hepatocellular coagulative necrosis increased slightly but steadily with age. Variably sized foci of cystic sinusoidal dilatation were seen in older age groups. This lesion appeared to result from hepatocellular atrophy and/or necrosis, with retention of the reticular framework to form the walls of the cystic spaces. A similar lesion has been described in old Wag/Rij rats (Boorman & Hollander, 1973). Eight rats with mononuclear cell leukemia had hepatic sinusoidal infiltration of leukemic cells. An apparently much higher incidence of hepatic infiltration was reported elsewhere (Moloney et al., 1970). One diaphragmatic hernia (presumably congenital), one reticulum cell sarcoma, and two cases of lymphosarcoma were seen.

The gastrointestinal tract was remarkably free of lesions (Table 11). There were six cases of focal glandular dilation of gastric mucosa, and 1 rat had focal chronic gastritis. Metastatic enteric mineralization occurred in 1 rat. Two rats had focal nonsuppurative sialoadenitis. One moribund rat had acute colitis. Mesenteric lipomas in 2 rats were the only form of neoplasia found.

The most frequent pancreatic lesion was islet cell adenoma which occurred in 9 rats (Table 7). A review of other studies indicated a much lower incidence than the 6% seen in this study (Rowlatt, 1967). This is probably because all tumors occurred in rats over 18 mo old, with the highest incidence (30%) occurring

at 30 or more months of age. The next most frequent lesion was pancreatic lobular atrophy, which occurred in 6 rats more than 18 mo old. This has been reported to be a common finding in the aging rat (Andrew, 1944; Berg, 1967). Acinar and ductal ectasia were found in a small number of rats without associated lobular atrophy. A few rats with evidence of previous hemorrhage, resolved pancreatitis, alpha cell degeneration, thrombosis, and lymphosarcoma were seen. Two rats had periarteritis nodosa of the pancreatico-duodenal artery which is a common location for this lesion (Berg & Harmison, 1955; Skold, 1961; Yang, 1965).

Reproductive System: Lesions of the testes and accessory sex glands were common (Tables 12 & 13). The earliest testicular change was epithelial degeneration of scattered seminiferous tubules in a 150-day-old rat. By 300 days, many testes had nodular interstitial (Leydig) cell hyperplasia. This appeared to precede interstitial cell neoplasia, since hyperplasia was not found after 520 days, and interstitial cell tumors began to appear at 510 days; tumors sharply increased in incidence in the 18- to 24-mo group and reached an incidence of 100% by 30 mo. A high incidence of this type of tumor in Fischer rats, but not other rat strains, has been noted by others (Davey & Moloney, 1970; Jacobs & Huseby, 1967). No metastases were seen. The only other type of tumor seen was papillary mesothelioma, arising from mesothelium of the tunica albuginea, in 3 rats. One

Table 11. Digestive System Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Focal gastric glandular dilatation	—	1(7.7) 1,-,-	1(5.0) 1,-,-	1(2.5) -,1,-	2(4.3) 2,-,-	1(6.7) 1,-,-	6(4.2) 5,1,-
Focal chronic sialoadenitis	—	—	—	1(2.5) -,1,-	1(2.1) 1,-,-	—	2(1.4) 1,1,-
Mesenteric lipoma	—	—	—	1(2.5) -,1,-	—	1(6.7) -,1,-	2(1.4) -,2,-
Focal chronic gastritis	—	—	—	—	1(2.1) -,1	—	1(0.7) -,1
Metastatic enteric mineralization	—	—	—	—	—	1(6.7) 1,-,-	1(0.7) 1,-,-
Acute colitis	—	—	—	—	—	1(6.7) -,1	1(0.7) -,1
No changes	9(100)	12(92.3)	19(95.0)	37(92.5)	43(91.5)	11(73.3)	131(91.0)
	13(91.0)	9,-,-	15,1,3	8,15,14	19,8,16	7,2,2	70,2,35

^athe numbers A (B) indicate: A = # of cases within the age group
B = % of cases in the age group
C,D,E = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

rat had epithelial hyperplasia of the ductus efferentes.

The incidence of testicular atrophy increased with age. Atrophy of the seminiferous tubules was first noted in 2 rats in the 6- to 12-mo group, then increased to 100% incidence by 18 mo. Two rats in the oldest age group had focal dystrophic mineralization of seminiferous tubules. The onset of interstitial cell tumors was coincidental with the onset of seminiferous tubular atrophy, but a few rats had testicular atrophy without tumors.

Concurrent with testicular atrophy, the seminal vesicles became shrunken with decreased secretory activity. One case of prostatic atrophy was found. Inflammatory lesions of the prostate, but not seminal vesicles, were common. Both nonsuppurative and suppurative lesions were found.

Central Nervous System: Lesions of the brain and spinal cord were present in only 1 rat before 18 mo (Table 14). The rat had protrusion of an intervertebral disk, with secondary degeneration of the spinal cord and resulting

Table 12. Testicular Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	19	35	42	15	133
	9,-,-	13,-,-	16,1,2	8,18,9	22,8,12	9,3,3	77,30,26
Atrophy of seminiferous tubules	—	2(15.4) 2,-,-	5(26.3) 4,-,1	28(80.0) 7,13,8	42(100) 22,8,12	15(100) 9,3,3	92(69.2) 44,24,24
Interstitial cell tumor	—	—	4(21.1) 3,-,1	28(80.0) 7,14,7	41(97.6) 22,7,12	15(100) 9,3,3	88(66.2) 41,24,23
Interstitial cell hyperplasia	—	3(23.1) 3,-,-	6(31.6) 5,1,-	—	—	—	9(6.8) 8,1,-
Mesothelioma	—	—	—	2(5.7) -,-,2	—	1(6.7) -,-,1	3(2.3) -,-,3
Dystrophic mineralization of seminiferous tubules	—	—	—	—	—	2(13.3) 1,-,1	2(1.5) 1,-,1
Hyperplasia, ductus efferentes	—	—	—	1(2.9) -,-,1	—	—	1(0.8) -,-,1
Degenerative change without atrophy	1(11.1) 1,-,-	—	—	—	—	—	1(0.8) 1,-,-
No changes	8(88.9) 8,-,-	9(69.2) 9,-,-	10(52.6) 9,-,1	4(11.4) 1,2,1	—	—	31(23.3) 27,2,2

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E
B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 13. Accessory Sex Gland Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	19	35	42	15	133
	9,-,-	13,-,-	16,1,2	8,18,9	22,8,12	9,3,3	77,30,26
Hyposecretion, seminal vesicles	—	1(7.7) 1,-,-	1(5.3) 1,-,-	19(54.3) 4,10,5	20(47.6) 10,3,7	10(66.7) 2,8,-	51(38.3) 18,21,12
Focal suppurative prostatitis	—	4(30.8) 4,-,-	9(47.4) 8,-,1	15(42.9) 2,9,4	13(31.0) 7,3,3	3(20.0) 2,1,-	44(33.1) 23,13,8
Focal chronic interstitial prostatitis	—	4(30.8) 4,-,-	5(26.3) 5,-,-	7(20.0) -6,1	7(16.7) 6,1,-	3(20.0) 2,1,-	26(19.5) 17,8,1
Prostatic atrophy	—	—	—	—	1(2.3) -,-,1	—	1(0.8) -,-,1
Focal chronic interstitial seminal vesiculitis	—	—	—	—	—	1(6.7) 1,-,-	1(0.8) 1,-,-
Focal suppurative seminal vesiculitis	—	—	—	—	—	1(6.7) 1,-,-	1(0.8) 1,-,-
No changes	9(100) 9,-,-	8(61.5) 8,-,-	9(47.4) 7,1,1	11(31.4) 3,5,3	16(38.1) 9,2,5	4(26.7) -,-,1,3	57(42.9) 36,9,12

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E
B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

posterior paresis. All other central nervous system lesions were restricted to the brain. Six rats with pituitary adenomas had secondary compression atrophy of overlying brain. Scattered single instances of hemorrhagic necrosis, ischemic malacia, atherosclerosis, and hemorrhage were present in other rats. Well defined, nonstaining vacuoles, adjacent to or within neurons or within the neuropil of the cerebellar white matter and brain stem were encountered with increasing age (Garner, Innes, & Nelson, 1967), and reached 80% incidence in

the oldest group. Two rats had posterior paralysis of undetermined cause. The cauda equina or peripheral nerves were not examined for radiculoneuropathy (Berg, Wolf, & Simms, 1962), but myelin degeneration was not seen in the upper lumbar spinal cord.

Musculoskeletal System: Lesions of the musculoskeletal system were rare and did not appear until after 18 mo, except in the rat with intervertebral disk protrusion (Tables 15 & 16). Focal muscular dystrophy was present in 2 rats, dystrophic mineralization occurred in a

Table 14. Central Nervous System Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	39	47	15	143
	9,-,-	13,-,-	16,1,3	8,17,14	22,8,17	9,3,3	77,29,37
Vacuolization	—	—	—	2(5.1) -,,-2	6(12.8) 5,-,1	12(80.0) 9,1,2	20(14.0) 14,1,5
Pressure atrophy, secondary to pituitary adenoma	—	—	—	3(7.7) 1,-,2	3(6.4) -,1,2	—	6(4.2) 1,1,4
Internal hydrocephalus	—	—	—	1(2.6) -,,-1	—	—	1(0.7) 1,-,1
Focal hemorrhagic necrosis	—	—	—	1(2.6) -,,-1	—	—	1(0.7) -,,-1
Ischemic necrosis	—	—	—	—	1(2.1) -,,-1	—	1(0.7) -,,-1
Atherosclerosis	—	—	—	—	1(2.1) -,,-1	—	1(0.7) -,,-1
Focal hemorrhage	—	—	—	—	1(2.1) -,,-1	—	1(0.7) -,,-1
Spinal cord degeneration (disk protrusion)	—	—	1(5.0) -,,-1	—	—	—	1(0.7) -,,-1
No changes	9(100) 9,-,-	13(100) 13,-,-	19(95.0) 16,1,2	34(87.2) 7,17,10	36(76.6) 17,7,12	3(20.0) -,2,1	114(79.7) 62,27,25

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 15. Muscle Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	46	15	143
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,16	9,3,3	77,30,36
Atrophy	—	—	—	—	—	4(26.7) 3,1,-	4(2.8) 3,1,-
Focal dystrophy	—	—	—	2(5.0) 1,-,1	—	—	2(1.4) 1,-,1
Focal mineralization	—	—	—	—	—	1(6.7) -,,-1	1(0.7) -,,-1
Rhabdomyosarcoma	—	—	—	1(2.5) 1,-,-	—	—	1(0.7) 1,-,-
Lymphosarcoma	—	—	—	—	1(2.2) -,,-1	—	1(0.7) -,,-1
Mononuclear cell leukemic infiltration	—	—	—	—	1(2.2) -,,-1	—	1(0.7) -,,-1
No changes	9(100) 9,-,-	13(100) 13,-,-	20(100) 16,1,3	37(92.5) 6,18,13	44(95.6) 22,8,14	10(66.7) 6,2,2	133(93.0) 72,29,32

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

few muscle fibers of the quadriceps femoris of 1 rat, and 4 rats had diffuse muscular atrophy. One case each of subscapular rhabdomyosarcoma, lymphosarcoma infiltration, and mononuclear cell leukemic infiltration was found.

Several skeletal lesions occurred in addition to the intervertebral disk protrusion previously discussed. Two rats had mild proliferative synovitis of the knee, and 2 rats with end-stage nephropathy had fibrous osteodystrophy.

Bone marrow lesions included three cases of myeloid or erythroid hyperplasia and focal areas of myelofibrosis in 2 rats. Nine of 23 rats with mononuclear cell leukemia had bone marrow involvement. Epiphyseal plates (distal femoral and proximal tibial) remained open for the life-span of the rats.

Ocular Lesions: Lesions of the eye and surrounding structures were uncommon (Table 17). Focal nonsuppurative Harderian dacryo-

Table 16. Bone Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	46	15	143
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,16	9,3,3	77,30,36
Mononuclear cell leukemic bone marrow infiltration	—	—	—	3(7.5) -,,-,3	4(8.7) 2,-,-,2	2(13.3) 1,-,-,1	9(6.3) 3,-,-,6
Bone marrow hyperplasia	—	—	—	1(2.5) -,1,-	1(2.2) 1,-,-	1(6.7) -,,-,1	3(2.1) 1,1,1
Focal myelofibrosis	—	—	—	—	—	2(13.3) -,1,1	2(1.4) -,1,1
Fibrous osteodystrophy	—	—	—	—	—	2(13.3) 2,-,-	2(1.4) 2,-,-
Chronic proliferative synovitis knee joint	—	—	—	1(2.5) -,1,-	1(2.2) -,,-,1	—	2(1.4) -,1,1
Protrusion of intervertebral disk	—	—	1(5.0) -,,-,1	—	—	—	1(0.7) -,,-,1
No changes	9(100) 9,-,-	13(100) 13,-,-	19(95.0) 16,1,2	35(87.5) 8,16,11	41(89.1) 19,8,14	9(60.0) 6,2,1	126(88.1) 71,27,28

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

Table 17. Ocular Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	39	46	15	142
	9,-,-	13,-,-	16,1,3	8,18,13	22,8,16	9,3,3	77,30,35
Nuclear cataract	—	—	—	—	1(2.2) -,,-,1	4(26.7) 3,1,-	5(3.5) 3,1,1
Chronic harderian adenitis	—	—	—	1(2.6) -,1,-	—	3(20.0) 3,-,-	4(2.8) 3,1,-
Chronic meibomian adenitis	—	2(15.4) 2,-,-	2(10.0) 2,-,-	—	—	—	4(2.8) 4,-,-
Keratitis	—	—	—	—	1(2.2) -,1,-	3(20.0) 2,1,-	4(2.8) 2,2,-
Focal scleral mineralization	—	—	—	—	4(8.7) 1,-,3	—	4(2.8) 1,-,3
Retinal degeneration	—	—	—	1(2.6) 1,-,-	1(2.2) -,,-,1	1(6.7) 1,-,-	3(2.1) 2,-,1
Atherosclerosis	—	—	—	2(5.1) 1,1,-	—	—	2(1.4) 1,1,-
Panophthalmitis	—	—	—	—	1(2.2) -,1,-	—	1(0.7) -,1,-
No changes	9(100) 9,-,-	11(84.6) 11,-,-	18(90.0) 14,1,3	35(89.7) 6,16,13	39(84.8) 21,6,12	7(46.7) 3,2,2	119(83.8) 64,25,30

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E B = % of cases in the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

adenitis occurred in 4 rats, and 4 rats had similar changes in the Meibomian glands. One rat had suppurative panophthalmitis, and 4 rats had chronic keratitis. Two rats had atherosclerosis of ocular arteries, 4 had focal scleral mineralization, 5 had nuclear cataracts, and 3 had diffuse retinal degeneration (Dowling & Sidman, 1962).

Skin and Adnexae: There was a moderate incidence of lesions in the skin and cutaneous adnexae, most of which were neoplasms which occurred in middle and older age groups (Table 18).

With the exception of five epidermal inclusion cysts and one sebaceous nevus, the remaining lesions were neoplasms, which began to appear at 300 days. Among the most frequent tumors were preputial gland sebaceous adenomas with squamous differentiation. They were often cystic and contained desquamated epithelial debris. The tumors had a fine connective tissue matrix, a thick collagenous capsule, and were invariably benign. Fibromas occurred in equal frequency. Most were subcutaneous, but one was intradermal. They were highly collagenous, hypocellular,

and often mineralized. Three rats had subcutaneous lipomas. Tumors of the auditory sebaceous glands (Zymbal's glands) occurred in 4 rats. A similar low spontaneous incidence of this tumor type has been reported by others (Schardein, & Kaump, 1966; Snell, 1965; Tannenbaum, Vesselinovich, Maltoni, & Mitchell, 1962). These tumors were all sebaceous with squamous differentiation. The squamous component in 1 rat gave rise to a squamous cell carcinoma with pulmonary metastases. Squamous papillomas were found in 2 rats, one on the lip and the other on the back. Three rats had basal cell tumors, 1 had squamous and follicular differentiation, and 2 had trichoepitheliomas. A single rat had a subcutaneous capillary angioma.

Nonneoplastic lesions included 1 rat with focal suppurative epidermitis, due to self-inflicted trauma of the eyelid overlying a chronic Meibomian adenitis, and 1 rat with acute necrotizing posthitis.

Mammary lesions (Table 19) consisted of nine cases of gynecomastia, one case of pyogranulomatous mastitis, and three cases of adenofibromatosis. Only 4 rats with gynec-

Table 18. Skin and Adenexal Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	37	46	15	140
	9,-,-	13,-,-	16,1,3	8,18,11	22,8,16	9,3,3	77,30,33
Prepuccial adenoma	—	1(7.7) 1,-,-	1(5.0) -,1,-	3(8.1) -,3,-	4(8.7) 1,1,2	1(6.7) 1,-,-	10(7.1) 3,5,2
Fibroma, subcutaneous	—	—	—	6(16.2) -,4,2	1(2.2) -,1,1	2(13.3) 1,-,1	10(7.1) 1,5,4
dermal	—	—	—	1(2.7) -,1,-	—	—	—
Epidermal inclusion cyst	—	—	—	1(2.7) -,1,-	4(8.7) 2,-,2	—	5(3.6) 2,1,2
Auditory sebaceous tumor	—	—	—	2(5.4) 1,1,-	—	2 ^b (13.3) 2,-,-	4(2.9) 3,1,-
Basal cell tumors	—	—	1(5.0) 1,-,-	2(5.4) 2,-,-	—	—	3(2.1) 3,-,-
Lipoma subcutaneous	—	—	1(5.0) -,1,-	1(2.7) -,1,-	1(2.2) 1,-,-	—	3(2.1) 1,1,1
Squamous papilloma	—	—	—	—	1(2.2) -,1,1	1(6.7) 1,-,-	2(1.4) 1,-,1
Focal suppurative epidermitis	—	1(7.7) 1,-,-	—	—	—	—	1(0.7) 1,-,-
Necrotizing posthitis	—	—	1(5.0) -,1,-	—	—	—	1(0.7) -,1,-
Angioma, subcutaneous	—	—	—	—	1(2.2) -,1,-	—	1(0.7) -,1,-
No changes	9(100) 9,-,-	11(84.6) 11,-,-	17(85.0) 15,-,2	19(51.3) 5,7,7	31(67.4) 16,5,10	10(66.7) 5,3,2	97(69.3) 61,15,21

^athe numbers A (B) indicate: A = # of cases within the age group
C,D,E
B = % of cases within the age group
C = # of randomly selected rats with lesion
D = # of "unhealthy" rats with lesion
E = # of dead or moribund rats with lesion

^b1 with transformation to squamous cell carcinoma & metastases to lung.

mastia had concurrent pituitary lesions, despite evidence of association of these 2 lesions (Ito et al., 1972).

Clinical chemistry. — Significant age-related changes in total protein, albumin, alpha-1

globulin, and cholesterol concentrations (Fig. 2-5 & Tables 20, 21) were observed. The same parameters had a significant relationship with the degree of chronic nephropathic change (Fig. 6-9 and Tables 20, 22). Interrelationships

Fig. 2. Significant relationship of increasing age to decreasing serum total protein levels in randomly selected rats ($p = 0.99$).

Fig. 3. Significant relationship of increasing age to decreasing serum albumin levels in randomly selected rats ($p = 0.99$).

Fig. 4. Significant relationship of increasing age to increasing serum alpha 1 globulin levels in randomly selected rats ($p = 0.99$).

Fig. 5. Significant relationship of increasing age to increasing serum cholesterol levels in randomly selected rats ($p = 0.99$).

Table 19. Mammary Gland Lesions.

Age (Months)	4-6	6-12	12-18	18-24	24-30	30-33	Total
Number of Rats	9 ^a	13	20	40	47	15	144
	9,-,-	13,-,-	16,1,3	8,18,14	22,8,17	9,3,3	77,30,37
Gynecomastia	—	1(7.7) 1,-,-	1(5.0) 1,-,-	3(7.5) -,3,-	3(6.4) -,3	1(6.7) 1,-,-	9(6.2) 3,3,3
Adenofibroma	—	—	—	—	2(4.3) 1,-,1	1(6.7) 1,-,-	3(2.1) 2,-,1
Pyogranulomatous mastitis	—	—	—	—	—	1 1,-,-	1(0.7) 1,-,-
No changes	9(100) 9,-,-	12(92.3) 12,-,-	19(95.0) 15,1,3	37(92.5) 8,15,14	42(89.4) 21,8,13	12(80.0) 6,3,3	131(91.0) 71,27,33

^athe numbers A (B) indicate: A = # of cases within the age group
 B = % of cases within the age group
 C = # of randomly selected rats with lesion
 D = # of "unhealthy" rats with lesion
 E = # of dead or moribund rats with lesion

Table 20. Serum Chemical Values of Randomly Selected Rats.

	4-6	6-12	12-18	18-24	24-30	30+
Total protein, gm/dl	8.9-9.4(6) ^a 9.1+/-0.2	6.7-9.8(11) 8.6+/-0.2	8.1-9.9(15) 8.8+/-0.1	7.7-9.9(8) 8.5+/-0.2	6.2-10.7(18) 8.1+/-0.3	6.1-7.2(6) 6.9+/-0.2
Albumin, gm/dl	3.7-4.8(6) 4.2+/-0.2	3.4-4.2(10) 3.8+/-0.1	1.5-3.8(15) 3.2+/-0.1	2.4-4.6(6) 3.3+/-0.3	2.6-5.8(9) 3.3+/-0.3	1.9-2.8(5) 2.5+/-0.2
alpha 1 globulin, gm/dl	1.8-2.1(6) 2.0+/-0.1	0.4-2.4(10) 1.5+/-0.3	1.14-3.0(15) 2.2+/-0.1	1.4-2.8(5) 2.3+/-0.3	1.1-3.2(9) 2.5+/-0.2	1.8-3.1(5) 2.2+/-0.2
alpha 2 globulin, gm/dl	0.9-1.3(6) 1.1+/-0.1	1.0-2.5(10) 1.7+/-0.2	0.9-1.5(15) 1.2+/-0.0	1.0-1.2(5) 1.0+/-0.0	0.3-1.5(9) 1.1+/-0.1	0.4-0.9(3) 0.7+/-0.1
beta globulin, gm/dl	1.1-1.6(6) 1.3+/-0.1	1.1-2.0(10) 1.4+/-0.1	1.1-2.64(15) 1.7+/-0.1	1.5-2.09(6) 1.8+/-0.1	1.0-2.2(9) 1.5+/-0.1	0.7-1.8(5) 1.3+/-0.2
gamma globulin, gm/dl	0.3-0.6(6) 0.4+/-0.05	0.3-0.8(10) 0.4+/-0.05	0.2-0.9(15) 0.5+/-0.1	0.2-0.8(6) 0.5+/-0.1	0.2-0.7(9) 0.5+/-0.1	0.3-0.5(5) 0.4+/-0.04
Sodium, mEq/L	104.0-135.5(6) 123.1+/-5.3	116.5-137.0(11) 128.4+/-1.8	126.0-137.5(15) 130.8+/-1.1	126.5-143.0(6) 135.1+/-2.2	113.0-154.0(16) 127.0+/-2.9	141.0-158.0(6) 151.5+/-2.4
Potassium, mEq/L	3.3-4.1(6) 3.7+/-0.1	3.3-10.2(11) 5.5+/-0.6	3.3-10.2(15) 4.3+/-0.2	3.8-8.2(6) 5.6+/-0.7	3.25-14.0(16) 5.6+/-0.7	3.4-7.2(6) 4.9+/-0.5
Urea nitrogen, mg/dl	—	—	—	—	21.0-32.0(5) 25.8+/-1.9	20.2-82.7(6) 35.7+/-9.7
Creatinine, mg/dl	0.66-1.00(6) 0.88+/-0.05	0.86-1.20(9) 0.97+/-0.03	0.68-1.60(14) 0.97+/-0.06	0.17-1.50(4) 0.83+/-0.28	0.17-1.60(13) 0.83+/-0.13	0.70-4.80(6) 1.52+/-0.66
Cholesterol, mg/dl	25.0-222.0(6) 123.9+/-31.9	62.0-200.0(10) 119.8+/-12.0	124.6-696.0(15) 246.8+/-37.6	36.0-195.0(5) 141.4+/-29.4	152.0-752.0(17) 311.4+/-41.7	175.3-281.4(6) 240.6+/-17.9

^athe numbers represented are:
 8.7-9.4 = range (6) = number rats
 9.1+/-0.2 = means+/- standard error of the mean

Table 21. Correlation Coefficients for Age vs. Serum Chemical Values.

	Number of Rats ^a	Correlation Coefficient ^b
Total protein	62	-0.56
Albumin	49	-0.53
alpha 1 globulin	51	+0.36
alpha 2 globulin	48	N.S.
beta globulin	51	N.S.
gamma globulin	51	N.S.
Sodium	60	N.S.
Potassium	60	N.S.
Creatinine	52	N.S.
Cholesterol	59	+0.36

^arandomly sampled rats only
^b = +/- 0.30, $p = 0.99$

Table 22. Correlation Coefficients for Index of Renal Disease vs. Serum Chemical Values.

	Number of Rats ^a	Correlation Coefficient ^b
Total protein	62	-0.50
Albumin	49	-0.45
alpha 1 globulin	48	+0.38
alpha 2 globulin	48	N.S.
beta globulin	51	N.S.
gamma globulin	51	N.S.
Sodium	59	N.S.
Potassium	60	N.S.
Creatinine	52	N.S.
Cholesterol	59	+0.47

^aRandomly sampled rats only
^b = +/- 0.30, $p = 0.99$

between serum constituents independent of age and renal disease were sought, but no relationships were found.

Total protein concentration progressively decreased with increasing age and renal lesion severity (Fig. 2 & 6). This change was due primarily to a progressive decrease in serum

albumin concentration (Fig. 3 & 7). This has previously been shown to be due to urinary loss associated with nephro-pathy rather than a decrease in serum half-life or rate of production (Salatka, Kresge, Harris, Edelstein, & Ove, 1971). Globulin concentration increased with age and renal disease due to increases in the

Fig. 6. Significant relationship of increasing severity of chronic nephropathy to decreasing serum total protein levels in randomly selected rats ($p = 0.99$).

Fig. 7. Significant relationship of increasing severity of chronic nephropathy to decreasing serum albumin levels in randomly selected rats ($p = 0.99$).

Fig. 8. Significant relationship of increasing severity of chronic nephropathy to increasing serum alpha 1 globulin levels in randomly selected rats ($p = 0.99$).

Fig. 9. Significant relationship of increasing severity of chronic nephropathy to increasing serum cholesterol levels in randomly selected rats ($p = 0.99$).

alpha-1 globulin fraction (Fig. 4 & 8). An increase in alpha globulin concentration with age has been reported (Uebel, 1956) but the mechanisms responsible for this change have not been established. Other studies with rats (Berg, 1965; Salatka, 1965, et al., 1971; Ubell, 1965) has shown a progressive increase in gamma globulin with age, but this was not seen in this study. Since serum levels of gamma globulin reflect primarily the host's immune response to antigenic stimuli, direct comparison of results from different studies is not possible.

Cholesterol levels in individual rats of all age groups were widely variable but there was a consistent increase with advancing age and renal disease (Fig. 5 & 9). Hypercholesterolemia has been reported in young rats with renal disease, suggesting a causal relationship (Berg, 1965). The mechanism for this change is unknown. The wide range of cholesterol values at each age group was an unexpected finding, since the rats in this study were an inbred strain in a reasonably controlled environment and fed the same diet.

Serum sodium and potassium were not significantly altered with age and severity of kidney disease. Creatinine, another index of renal impairment, showed no relationship to age or renal damage. Only 1 rat had an elevated creatinine of 2.3 mg/dl. Serum urea nitrogen was measured only in rats in the older age groups, which had more advanced pathology. Several rats had levels exceeding 40 mg/dl, up to 87 mg/dl in 1 animal. No correlation occurred between creatinine and urea nitrogen levels.

Microbiology. — All of the rats sampled throughout the course of the study were free of *Mycoplasma pulmonis*, *Salmonella*, ectoparasites, and endoparasites. Occasional rats were found to have *P. aeruginosa* in their nasopharynx and/or cecum, with no evidence of associated pathology. Other rodent pathogenic bacteria were not isolated from any of the rats.

Serology. — A consistent pattern of antibodies to PVM, Sendai virus, and MVM was noted at the onset of the study and persisted throughout the study. Approximately 90% reacted to PVM (1:20-1:320), 70% reacted to Sendai virus (1:10-1:80), and 30% reacted to MVM (1:20-1:80). A limited number (10%) reacted weakly to MHV (1:10-1:20), and even fewer reacted to RCV (1:10-1:20). Antibodies to MHV most probably represent cross reactivity to a rat coronavirus rather than to MHV (Bhatt, Percy, & Jonas, 1972; Parker, Cross,

& Rowe, 1970). The pattern was identical in all age groups with the exception of MVM, to which only rats greater than 18 mo of age reacted. No chemical or histological evidence of clinical infection was noted.

DISCUSSION

This study further characterized the Fischer 344 male rat through its natural life-span. In order to obtain meaningful life-span information, the environment was stabilized in every way practicable. All animals were housed behind a barrier to minimize possible disease outbreaks. Other factors, such as food, water, heating, ventilation, caging, and bedding were kept reasonably uniform throughout the study. With these controls, the 50% mean survival was 29 mo. Maximum survival to 35 mo was observed. Mortality was negligible until about 20 mo, at which time the curve dropped rapidly, with a shouldered slope.

Cause of death of animals found dead or moribund was not evaluated in detail, since cause was not always apparent. As a generalization, most deaths were related to neoplasia or advanced renal disease. Dead or dying rats often had lesions equal in severity to their living cohorts. Clinical chemical parameters of live aged rats showed renal compensation, despite the severity of the lesion. It seemed probable that many rats died acutely following decompensation, but the chemistries are not available to prove this presumption.

A commercial breeder was chosen to evaluate the feasibility of maintaining and shipping aged rats to scientists. The results clearly demonstrate that maintaining aged rats free of major overt infectious disease is possible, as monitored by microbiological, serological, and pathological methods. The study demonstrates the efficacy of barrier facilities. Of note was the absence of chronic respiratory disease, associated with *Mycoplasma pulmonis*. The facility remains in operation, free of *M. pulmonis*, 6 years after its establishment. The only contaminant since tabulating these data has been *Syphacia obvelata* (pinworm). Hundreds of rats have been shipped from this colony to investigators. No untoward effects have been observed or reported, indicating the practicality of raising aging animals at one location and distributing them to users. Others (Flynn, Poole, & Tyler, 1971) have provided detailed documentation that aged animals could be successfully shipped by air across the country.

Pathology data in this study should be interpreted with caution. There was a variety of pathology found, but all animals, including man, develop lesions with increasing age. These data cannot be compared directly with data on other rat strains, since other strains have not been examined as critically as the aging male F344 rat used in this study. The male Fischer rat appeared to have a relatively low incidence of malignant solid tumors, an extremely low incidence of pulmonary disease of any type, and a low prevalence of a wide range of benign tumors. Testicular Leydig cell tumors were in virtually all aged male Fischer rats. Nonneoplastic lesions were varied and found as expected in almost all organs with advancing age. Renal disease, as previously mentioned, was suspected as a contributor to the cause of death of many of the rats and was shown to affect serum chemical parameters with age. The hepatic bile ductal hyperplasia and sclerosis was interpreted as the result of an environmental insult rather than a natural progressive lesion associated with aging *per se*. Analysis of feed is in progress to detect the presence of a possible hepatotoxic component in the diet, and the lesion is being further studied.

SUMMARY

Pathology, microbiology, and selected serum chemistries were evaluated in 144 male Fischer rats from 4 to 33 mo of age. The rats were reared and maintained under barrier conditions, which successfully excluded the introduction of major infectious disease agents throughout the entire study, including *Mycoplasma pulmonis*. A wide variety of pathology was found and tabulated, and many lesions were found to increase in severity and incidence with age. Involution of the reproductive system, myocardial degeneration and fibrosis, chronic renal disease, sclerosing bile ductal hyperplasia, and chronic passive splenic congestion were the most frequent nonneoplastic lesions seen. Testicular interstitial cell tumors, mononuclear cell leukemias, and pituitary adenomas were the most frequent tumors found, with a large number of other types occurring in low incidence. There was a high correlation of renal disease severity with increasing age. Serum total protein and albumin decreased with age, while alpha-1 globulin and cholesterol increased. No changes were seen

in serum sodium, potassium, urea, creatinine, or other protein components.

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