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Chapter 4

Biology and Diseases of Rats

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I. INTRODUCTION

A. Origin and History

The diversity of research for which the laboratory rat is used is probably greater than that associated with any other animal. The laboratory rat is a descendent of the wild rat, *Rattus norvegicus,* which originated in Asia and reached Europe in the early 1700s. Wild and albino mutants were first used for experimental purposes in Europe in the mid-1800s and in the United States shortly before 1900. The Wistar Institute in Philadelphia was prominent in the development of the rat as a laboratory animal, for here originated many of the rat strains now used worldwide. Henry Donaldson and his colleagues at the Wistar Institute used these early rat strains for a variety of studies dealing with neuroanatomy, nutrition, endocrinology, genetics, and behavior. The history and evolution of the many rat strains used today have been recently summarized (Lindsey, 1979).

The most commonly used outbred rat stocks in North America are the Wistar, Sprague-Dawley, Long-Evans, and Holtzman. All are albino except the Long-Evans stock, which is usually marked with a black or gray hair coat over the shoulders and is sometimes referred to as a "hooded rat." There are numerous inbred and mutant rat strains, although the number is

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less than that in the mouse. Table I lists the more commonly used strains.

B. Sources and Nomenclature

There are a rather large number of commercial vendors of laboratory rats in the United States. Most of the stocks and strains mentioned above can be obtained from more than one source. Although the origin of an outbred stock, such as the Sprague-Dawley, may have been the same for a number of vendors, in many cases it has been 20 to 30 years since such a stock has been removed from its original breeding colony. Accordingly, the genotype of outbred stocks and inbred strains may vary among sources and be reflected by differences in data when multiple sources of rats are used. A standardized scheme of identifying stocks and strains of rats has been developed and is now used by nearly all commercial vendors. Moreover, it is important that authors correctly identify stocks and strains that are used in their research since the success in repeating the work in another laboratory may be dependent upon the genotype (source of the rat). Table II summarizes the standardized nomenclature for outbred stocks as developed by the International Committee on Laboratory Animals (ICLA). Table III contains the scheme for designating inbred strains of rats (National Institutes of Health, 1981). "Animals for Research" (National Academy of Sciences, 1979), a directory of sources for laboratory animals sold in the United States and Canada, lists all rodents according to standard nomenclature, and is a valuable aid in purchasing laboratory animals.

Table I

Commonly Used Strains

Inbred strains	Usefulness as models[a]
ACI	Congenital genitourinary anomalies, prostatic adenocarcinomas
BN (Brown-Norway)	Inducible, transplantable myeloid leukemia, hydronephrosis
BUF (Buffalo)	Spontaneous autoimmune thyroiditis, host for transplantable Morris hepatomas
F344 (Fischer 344)	Long-term xenobiotic toxicity, gerontology, esophageal and urinary bladder carcinoma
LEW (Lewis)	Multiple sclerosis, various experimentally induced autoimmune disease
SHR (spontaneous hypertensive rat)	Hypertension, myocardial infection
WF (Wistar-Furth)	Mononuclear cell leukemia
Mutant strains	**Characteristics**
Brattleboro	Diabetes insipidus (autosomal recessive)
Gunn	Jaundice, kernicterus (autosomal recessive)
Nude	T cell deficient (autosomal recessive)
Obese SHR	Type 4 hyperlipoproteinemia (autosomal recessive)

[a]National Institutes of Health (1981).

Table II

Nomenclature for Outbred Stocks

1. Letters preceding the colon designate the supplier/breeder code consisting of a capital and two or three lowercase letters
2. Capital letters following the colon are used by a breeder to identify his stock
3. Letters in parentheses denote origin of stock
4. Subscript symbols indicate rearing by means other than natural mother (f, fostered; fh, fostered by hand)

C. Housing

Commercial production of rats has markedly changed since the 1960s due to the development of hysterectomy-derived and barrier-maintained breeding colonies. Prior to the application of this technology to production colonies, infectious diseases were ubiquitous in rats from most sources. Today, vendors can be selected who offer pathogen-defined animals for most stocks and strains. Concomitant with changes in commercial sources of rats are the major advances made in the design and construction of institutional animal resources and husbandry practices within them. Optimum housing of rats today includes provisions for quarantining and isolation of animals according to vendor subpopulations that have a similar microbial flora.

There are various levels of sophistication to provide barriers to the spread of infections in rat colonies. Since many rat pathogens are spread by aerosol, ventilation control is very important. Nonrecirculating room air or high-efficiency particulate air (HEPA)-filtered air has become a design standard in modern animal facilities. As discussed in Chapter 17, clean/contaminated corridor-designed facilities aid in containment against the spread of pathogens by aerosol, personnel,

Table III

Nomenclature for Inbred Rats

1. The strain designation is given in capital letters followed by a slash
2. The substrain designation follows the slash and is given as numbers or as individual or company codes. Numbers are used to denote substrains that were derived from a common strain but separated before the completion of inbreeding
3. Subscript symbols indicate rearing by means other than natural mother

and contaminated equipment. A more complete barrier system may include an entry area in which incoming supplies and equipment are sterilized and in which personnel shower and don sterile clothing and filter masks before entering animal rooms. More recently, laminar-flow (mass air displacement) rooms and mobile units have become popular because they can be incorporated in existing buildings that lack design characteristics mentioned above.

Environmental control in rat rooms is important to the comfort and health of the animals, as well as to the consistency of data derived from the rats. Room temperatures between 72 and 76°F are desirable, and the relative humidity should range between 40 and 60%. Daily fluctuations in temperature and humidity act as significant stressors. These fluctuations may be associated with the environmental control system of a building or may be induced by procedures such as cleaning floors with a water hose or high pressure sprayer. Twenty-four-hour temperature/humidity recorders are useful in detecting changes in environmental conditions. Light intensity should be evenly distributed to all animals within a room. Seventy-five to 125 fc have often been suggested as an optimal range for light intensity. However, recent evidence indicates that this intensity can induce retinal degeneration in albino rats (Anver and Cohen, 1979). Light-timing devices are a convenient means to provide desired day/night cycles such as 12–12 or 14–10 hr.

Caution should be exercised in the use of insecticides and air-deodorizing chemicals, since some have been shown to induce hepatic microsomal enzymes in rats. Accordingly, their use in animal rooms is not usually recommended (Baker *et al.*, 1979a).

Rats can be housed in either wire- or solid-bottom cages. Wire-bottom cages are more frequently used since they are less labor-intensive. Frequency of cage and litter pan changing is a function of animal density. Solid-bottom cages should be sanitized two to three times per week, while wire-bottom cages should be sanitized on a weekly or biweekly schedule with litter pans changed two or three times per week. Feed should be contained in hoppers. Either automatic systems or bottles are satisfactory for providing water to rats. Some caution is necessary when using automatic systems, since weanling and newly arrived rats may not drink initially from such devices. To avoid undesirable microbial contamination, water bottles should be sanitized before they are refilled and automatic systems should be drained and flushed when racks are sanitized. Acidification of water to a pH of 2.5 to 2.8 or chlorination at 8 to 12 ppm will control *Pseudomonas aeruginosa* contamination of water (Weisbroth, 1979). However, this treatment is not necessary for immunocompetent animals. Wood shavings or chips are the most commonly used contact bedding materials. Hardwoods are preferred to softwoods, since the latter are capable of inducing hepatic microsomal enzymes (Baker *et al.*, 1979a).

II. BIOLOGY

A. Anatomy

This section summarizes some of the anatomical characteristics of the rat with emphasis on characteristics that are unique. The reader is advised to refer elsewhere in the literature for comprehensive descriptions (Bivin *et al.*, 1979; Caster *et al.*, 1956; Hebel and Stromberg, 1972; Smith and Calhoun, 1972; Zeman and Innes, 1963).

1. Digestive System

The rat dental formula is 2(I 1/1, C 0/0, PM 0/0, M 3/3) = 16. The incisors are well developed and grow continuously. The rat lacks tonsils and water taste receptors.

The major pairs of salivary glands are the parotid, submandibular (submaxillary), and sublingual. The parotid gland is a serous gland consisting of three to four lobes and is located ventrolaterally from the caudal border of the mandible to the clavicle. The submandibular glands are mixed glands located ventrally between the caudal border of the mandibles and the thoracic inlet. The sublingual glands are mucous glands and are much smaller than the parotid and submandibular glands. They are located at the rostral pole of the submandibular glands to which they are closely associated. Brown fat deposits are present in the ventral cervical region. These multilocular deposits are well demarcated and can be confused with salivary glands or lymph nodes.

The stomach of the rat is divided into two parts; the forestomach (nonglandular) and the corpus (glandular). The two portions are separated by a limiting ridge. The esophagus enters at the lesser curvature of the stomach through a fold of the limiting ridge. This fold is responsible for the inability of the rat to vomit. The forestomach, which is thinner than the corpus, is linked with an epithelium similar to that of the esophagus and extends from the cardia to a narrow band of cardiac glands at the junction of the glandular portion.

The small intestine is composed of the duodenum (10 cm), jejunum (100 cm), and ileum (3 cm). The cecum is a thin-walled, comma-shaped pouch that has a prominent lymphoid mass in its apical portion. The colon is composed of the ascending colon, with prominent oblique mucosal ridges, transverse and descending colons, with longitudinal mucosal folds; followed by a short rectum that is confined to the pelvic canal.

The liver has four major lobes (median, right lateral, left, and caudate) and is capable of regeneration subsequent to partial hepatectomy. The rat has no gallbladder. The bile ducts from each lobe form the common bile duct that enters the duodenum 25 mm from the pyloric sphincter.

The pancreas is a lobulated, diffuse organ that extends from

the duodenal loop to the gastrosplenic omentum. It can be differentiated from adjacent adipose tissue by its darker color and firmer consistency. Up to 40 excretory ducts fuse into 2–8 large ducts, which empty into the common bile duct.

2. Respiratory System

The nasal cavity is not markedly different from that of other mammals. The rat has a maxillary recess (sinus) located between the maxillary bone and the lateral lamina of the ethmoid bone. The recess contains the lateral nasal gland (Steno's gland) that secretes a watery product that is discharged at the rostral end of the nasal turbinate. It has been postulated that the nonviscous secretion contributes to the humidification of inspired air and acts to regulate the viscosity of the mucous layer overlying the nasal epithelium.

The left lung has one large lobe, and the right lung is divided into four lobes (cranial, middle, accessory, and caudal). The pulmonary vein in the rat has cardiac striated muscle fibers within its wall that are contiguous with those in the heart. The rat does not have an adrenergic nerve supply to the bronchial musculature, and bronchoconstriction is controlled by vagal tone. Unlike the guinea pig, the rat lung has a low concentration of histamine (Bivin *et al.*, 1979).

3. Cardiovascular System

The heart and peripheral circulation in the rat differ little from that of other mammals. The blood supply to the heart is derived from both coronary and extracoronary arteries. The latter arise from the internal and subclavian arteries.

4. Genitourinary Systems

The right kidney, which is more craniad than the left, has its cranial pole at the L_1 vertebra and its caudal pole at the level of L_3. The rat kidney is unipapillate as are kidneys of other rodents, lagomorphs, and insectivores. Having only one papillus and calyx makes the rat useful for studies in which cannulization of the kidney is done. The presence of superficial nephrons in the renal cortex has made the rat widely used as a model for studying nephron transport in an *in vivo* micropuncture system.

The male reproductive system has a number of highly developed accessory sex glands. These include large seminal vesicles, a bulbourethral gland, and a prostate gland composed of the coagulation gland (dorsocranial lobe) and ventral and dorsolateral lobes. The inguinal canal remains open throughout the life of a rat and testes descend initially by 40 days of age.

The female rat has a bicornate uterus that is classified as the duplex-type because the lumina of the uterine horns are completely separate with paired ossa uteri and cervices. The female urethra does not communicate with the vagina or vulva, but rather exits at the base of the clitoris.

5. Central Nervous System

The brain of the rat has very large olfactory bulbs and a nonconvoluted cerebrum. The hypophysis is behind the optic chiasma and is attached to the base of the brain by a thin hollow stalk, the infundibulum. The blood supply to the brain is from the internal carotid and vertebral arteries. Blood leaves the brain via a system of sinuses that are enclosed in the dura mater. The ventricular system is similar to that of other animals, but the rat lacks a foramen of Magendie.

B. Normal Physiological Values

It must be recognized that many of the normal values determined for a specific group of rats may be accurate for only that rat stock/strain, source, and conditions under which they are held. Selected physiological, hematological, and clinical biochemical parameters are listed in Tables IV–VII. More complete information on biological values is available (Mitruka and Rawnsley, 1977; Ringler and Dabich, 1979).

C. Nutrition

Nutritionally adequate diets are readily available from commercial sources. These standard rations are quite satisfactory for most applications. However, for some types of experimentation there are factors, other than nutritional adequacy, which must be considered. The nutrient composition of diets and the contamination of feed components by mycotoxins, antibiotics, synthetic estrogens, heavy metals, and insecticides may have a profound impact on many studies. For instance, caloric intake and the percent of fat and protein in the diet of rats influence the incidence of neoplasia (Altman and Goodman, 1979). Similarly, various contaminants have an adverse effect on data from toxicologic, gerontological, and reproductive studies. Standard commercial diets are formulated from natural ingredients and will vary in nutrient composition on a batch-to-batch basis due to differences in type and quality of ingredients used. Commercial makers of rodent feeds take precautions to preclude the presence of contaminants in feeds, but only a few products have a defined profile of maximal levels of heavy metals, aflatoxins, chlorinated hydrocarbons, and organophosphates.

For some investigative purposes, feeds formulated with refined ingredients (purified diets) or with chemically defined compounds are useful when control of nutrient concentrations is essential (National Research Council, 1978). These diets are, however, too expensive for general use.

Table IV

Selected Normative Data[a]

Adult weight	
Male	300–400 gm
Female	250–300 gm
Life span	2.5–3 years
Body temperature	37.5°C
Basal metabolism rate (400 gm rat)	35 kcal/24 hr
Chromosome number (diploid)	42
Puberty	50 ± 10 days
Gestation	21–23 days
Litter size	8–14
Birth weight	5–6 gm
Eyes open	10–12 days
Weaning	21 days
Food consumption/24 hr	5 gm/100 gm body weight
Water consumption/24 hr	8–11 ml/100 gm body weight
Cardiovascular	
Arterial blood pressure	
Mean systolic	116 mm Hg
Mean diastolic	90 mm Hg
Heart rate	300–500 beats/min
Cardiac output	50 ml/min
Blood volume	6 ml/100 gm body weight
Respiratory	
Respirations/min	85
Tidal volume	1.5 ml
Alveolar surface area (400 gm rat)	7.5 m^2
Renal	
Urine volume/24 hr	5.5 ml/100 gm body weight
Na^+ excretion/24 hr	1.63 mEq/100 gm body weight
K^+ excretion/24 hr	0.83 mEq/100 gm body weight
Urine osmolarity	1659 mOsm/kg of H_2O
Urine pH	7.3–8.5
Urine specific gravity	1.04–1.07

[a]Data from Baker *et al.* (1979b) and Bivin *et al.* (1979).

Rats are commonly fed *ad libitum*, and food intake will vary according to requirements for growth, gestation, and lactation. The nutritive requirements for the rat are listed in Table VIII.

The duration of storage and the temperature at which feeds are stored prior to use effect the nutritive quality of diets. Commercial diets are formulated to have a shelf life of up to 6 months. However, storage in a hot or damp environment will reduce this shelf-life. To help assure that only fresh diets are used, products should be used which have milling dates identified on their containers (see Chapter 17).

D. Biology of Reproduction

1. Reproductive Physiology

Sexual maturity occurs between 6 and 8 weeks for both sexes, although the onset of first estrus in females occurs at about 5 weeks. The vagina opens between 34 and 109 days, and the testes descend between 15 and 51 days, although they remain fully retractable in adults. Rats ovulate spontaneously, but ovulation can also be induced by forced coitus during nonestrous intervals. Vaginal stimulation during mating is important in rat reproductive physiology. The more often a male inserts his penis into the vagina prior to ejaculation, the greater the probability of a resulting pregnancy. However, natural or artificial stimulation of the vagina within 15 min of a first mating will abrogate pregnancy from the first mating by inhibition of sperm transport. A 12-hr estrous period recurs every 4 to 5 days and after parturition, without seasonal variation. Estrus can be suppressed when females are housed in groups and synchronized in the presence of a male or its excreta (Whitten effect), but this effect is not as pronounced as in the mouse. Female fertility wanes at 600 to 650 days, but estrous cycles may continue through 32 months. Male fertility is lost between 16 and 20 months. Fertility of both sexes is generally regarded as maximal between 100 and 300 days of age (Adler and Zoluth, 1970; Baker, 1979; Farris, 1963; Lane-Petter, 1972; Leathem, 1979).

Males will mount estrous females numerous times with one or two rapid ejaculations in the course of 15 to 20 minutes. Ejaculated semen coagulates, forming a copulatory plug that remains in the distal vagina for a few hours, after which time it dissolves or is extruded. Copulation is usually nocturnal. Duration of gestation varies with strain, age, litter size, and other variables, and ranges from 19 to 23 days, with an average of 21 or 22 days. Primiparous females tend to have a slightly longer gestation than multiparous females (Farris, 1963).

2. Detection of Estrus and Pregnancy

Estrus can be detected in a number of ways. Females in estrus are hyperactive and brace themselves when touched. Their ears quiver when they are stroked on the head or back, and stimulation of the pelvic region induces lordosis (Farris, 1963). The vulva becomes swollen, and the vagina becomes dry in contrast to the moist pink wall during metestrus or diestrus. As proestrus occurs (approximately 12 hr), smears of vaginal cells contain nucleated epithelium, leukocytes, and occasional cornified cells. Estrus (approximately 12 hr) begins with about 75% nucleated and 25% cornified cells, with cornified cells predominating as estrus continues. Metestrus follows (approximately 21 hr) with large numbers of leukocytes and cornified cells, which form abundant caseous vaginal detritus. Metestrus is characterized by the presence of large flat nucleated (pavement) cells. Diestrus persists for approximately 57 hr (Baker, 1979; Farris, 1963).

Breeding dates can be established by examination of vaginal swabs for spermatozoa or examining the distal vagina or cage pan for copulatory plugs. Timed pregnancies are best achieved by placing the female in the male's cage in the afternoon and examining her for a plug or spermatozoa the following morning.

Table V

Hematological Parameters in the Rat[a,b]

Age (weeks)	Stock/strain	Erythrocyte parameters											
		Erythrocyte ($\times 10^6/\mu l$)		PCV[c] (%)		Hemoglobin (gm/dl)		MCV[d] (fl)		MCHC[e] (%)		Reticulocytes (%)	
		M	F	M	F	M	F	M	F	M	F	M	F
6	Hsd:SD(SD)BR	6.31	5.93	42	39	13.9	13.6	ND		ND		ND	
8	Crl:CD(SD)BR	7.69	7.25	41.7	40.7	15.6	14.9	54.5	55.6	40.1	40.8	ND	
16	Crl:CD(SD)BR	8.27	7.62	40.5	39.4	16.0	15.7	49.8	51.9	39.5	40.7	ND	
26	Hsd:SC(SD)BR	5.63	6.36	40	44	13.7	15.6	ND		ND		ND	
26	CDF(F344)/CrlBR	9.17	9.03	48.1	49.3	17.5	16.9	52.6	54.6	36.5	34.4	1.66	0.81
40	Crl:CD(SD)BR	8.45	7.49	41.1	38.5	16.1	15.4	48.4	51.2	38.9	39.9	ND	
52	CDF(F344)/CrlBR	9.13	8.41	47.2	46.8	16.2	15.7	51.8	55.9	34.3	33.5	0.46	1.69
78	CDF(F344)/CrlBR	9.60	8.32	54.5	46.7	18.9	16.3	56.8	56.3	34.7	35.1	1.99	1.80
104	CDF(F344)/CrlBR	9.26	8.20	57.8	45.8	19.5	15.1	62.4	55.9	33.7	32.9	3.08	1.25

Age (weeks)	Stock	Leukocyte parameters											
		WBC ($\times 10^3$ μl)		Neutrophil[d]		Lymphocyte[f]		Monocyte[f]		Eosinophil[f]		Basophil[d]	
		M	F	M	F	M	F	M	F	M	F	M	F
6	Hsd:SD(SD)BR	9.8	6.3	16	18	80	75	3	4	1	3	0	0
8	Crl:CD(SD)BR	17.2	10.0	15.7	19.3	81.2	77.7	2.2	1.9	0.7	1.0	0.2	0.3
16	Crl:CD(SD)BR	14.9	10.3	13.9	14.1	82.8	83.1	2.4	2.0	0.8	0.8	0	0
	CDF(F344)/CrlBR	10.2	13.8	23.8	31.7	73.4	66.0	2.3	1.8	0.9	0.5	0	0

[a]T. E. Hamm, unpublished data.
[b]Ringler and Dabich (1979).
[c]PCV, packed cell volume.
[d]MCV, mean corpuscular volume.
[e]MCHC, mean corpuscular hemoglobin content.
[f]Number per 100 cells counted.

Table VI

Clinical Chemistry Parameters in the Rat[a,b]

Age (weeks)	Stock/strain	Fasting blood glucose (mg/dl)		BUN[c] (mg/dl)		SGPT[d] (IU/liter)		ALK PHOS[e] (IU/liter)	
		M	F	M	F	M	F	M	F
6	Hsd:SD(SD)BR	163	169	8	8	89	92	ND	
26	Hsd:SD(SD)BR	219	217	7	6	75	61	ND	
26	CDF(F344)/CrlBR	ND		17.9	16.1	28.6	19.8	63.6	40.0
30	Crl:CD(SD)BR	134		15.4		ND		22	16
52	CDF(F344)/CrlBR	ND		22.9	20.0	39.5	26.5	95.9	58.5
78	CDF(F344)/CrlBR	ND		16.6	18.0	24.0	23.5	53.4	59.5
104	CDF(F344)/CrlBR	ND		23.9	19.3	21.2	21.2	66.7	53.9

[a]T. E. Hamm, unpublished data.
[b]Ringler and Dabich (1979).
[c]BUN, blood urea nitrogen.
[d]SGPT, serum glutamic pyruvic transaminase.
[e]ALK PHOS, alkaline phosphatase.

Table VII

Growth Rates in Rats[a]

Weight (gm)	Crl:CD(SD)BR (days old)		Hla:(SD)BR (days old)		Crl:(LE)BR (days old)		Crl:(WI)BR (days old)		Hla:(WI)BR (days old)		CDF(F344)/ CrlBR (days old)		NIH:(F344)/ HlaBR (days old)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
51–75	22–26	22–30	21–26	21–27	25–29	25–30	22–30	21–25	21–25	21–26	29–35	28–32	29–35	32–39
76–100	27–30	31–35	26–30	27–32	30–33	31–34	31–35	26–30	25–28	26–30	36–42	33–39	35–42	39–46
101–125	31–35	36–40	30–33	32–35	34–37	35–40	36–39	31–35	28–32	30–33	43–49	40–46	42–48	46–58
126–150	36–42	41–47	33–37	35–38	38–41	41–48	40–43	36–42	32–35	33–36	50–56	47–58	48–56	58–70
151–175	43–46	48–54	37–40	38–43	42–45	49–55	44–48	43–48	35–37	36–41	57–63	59–70	56–63	
176–200	47–50	55–65	40–43	43–51	46–49	56–70	49–51	49–57	37–40	41–48	64–69		63–69	
201–225	51–55	66–75	43–47	51–60	50–53	71–90	52–56	58–70	40–43	48–53			69–75	
226–250	56–60	76–84	47–50	60–70	54–58	91–107	57–61	71–85	43–46	53–62				
251–275	61–65		50–52		59–66		62–68	86–98	46–48					
276–300	66–70		52–55		67–76		69–77		48–51					
301–325	71–74		55–60		77–88		78–85		51–56					
326–350	75–80		60–64		89–101		86–94		56–59					
351–375	81–87		64–70		102–117		95–103		59–63					

[a]Adapted from vendor data.

Abdominal enlargement becomes evident at about 2 weeks. Pseudopregnancy is rare (Lane-Petter, 1972).

3. Husbandry Needs

Rats reproduce successfully under a variety of conditions, but husbandry practices can significantly influence fecundity. Rats can be bred as monogamous pairs, taking advantage of postpartem estrus for maximal breeding efficiency. Polygamous breeding is more economical, since only one male can be kept with 6 to 9 females. Females are often removed to a separate cage prior to whelping, since they may not tolerate other females in the cage while nursing. They will tolerate their mates, however. Females with litters do best on clean dust-free wood shavings in solid-bottom cages. Due to heat regulation, pups neither thrive in overly spacious cages with wide flutuations in ambient temperature, nor in overly crowded cages where they cannot dissipate heat. The recommended cage floor area for a female and her litter is 150 in.^{2}. Ambient room temperature and humidity should be within the acceptable range with minimal fluctuation. High ambient temperature can cause male infertility (Baker, 1979; Baker *et al.*, 1979a; Lane-Petter, 1972).

The rat estrous cycle is particularly sensitive to variations in light. Daily lighting at an average of 100 fc with a spectrum approximating natural light for 12 to 16 hr is best for breeding. Constant light for as few as 3 days may induce persistent estrus, hyperestrogenism, polycystic ovaries and endometrial hypertrophy or metaplasia (Baker *et al.*, 1979a; Gralla, 1981).

Nutrition may also affect reproductive performance. Requirements for certain components are increased during pregnancy and growth, but overfeeding is deleterious. Caloric restriction may actually improve fertility and possibly reproductive life of the female (Leathem, 1979). Excess dietary protein can adversely affect female sexual development. Vitamin deficiencies can cause infertility, particularly those vitamins (A, E, riboflavin, and thiamin) that are most labile to autoclaving or deterioration (Baker, 1979).

4. Parturition

It is not necessary to add nesting material to bedding for successful breeding, but rats will utilize it if offered. Shredded paper or cotton nesting material will be readily accepted and used by prepartem and nursing dams. Parturition is heralded by pronounced postural stretching and rear leg extension. A vaginal discharge may be noted $1\frac{1}{2}$–4 hr prepartum. Parturition is usually complete in 1 or 2 hr, but can range from a few minutes to several hours depending on litter size. Dystocia is exceedingly rare. Litters average between 6 and 12 pups, with highest fecundity through the sixth litter. Inbred rats tend to produce smaller litters. Although infrequent, cannibalism is most apt to occur with nervous or primiparous females subjected to stress (Farris, 1963; Lane-Petter, 1972; Leathem, 1979).

5. Early Neonatal Development

The neonate weighs about $5\frac{1}{2}$ gm, depending on litter size, sex, strain, and physical condition of the dam. Pups are born hairless, blind, with closed ears, undeveloped limbs, and short

Table VIII

Nutrient Requirements of Rats[a]

Nutrient	Concentration in a diet[b]	
	Growth, gestation, or lactation	Maintenance
Protein (as ideal protein)	12.00%	4.20%
Fat[c]	5.00%	5.00%
Digestible energy	3800.00 kcal/gm	3800.00 kcal/gm
L-Amino acids		
Arginine	0.60%	—
Asparagine	0.40%	—
Glutamic acid	4.00%	—
Histidine	0.30%	0.08%
Isoleucine	0.50%	0.31%
Leucine	0.75%	0.18%
Lysine	0.70%	0.11%
Methionine	0.60[d]	0.23%
Phenylalanine-tyrosine	0.80[e]	0.18%
Proline	0.40%	—
Threonine	0.50%	0.18%
Tryptophan	0.15%	0.05%
Valine	0.60%	0.23%
Nonessential[f]	0.59%	0.48%
Minerals		
Calcium	0.50%	
Chloride	0.05%	
Magnesium	0.04%	
Phosphorus	0.40%	
Potassium	0.36%	
Sodium	0.05%	
Sulfur	0.03%	
Chromium	0.30 mg/kg	
Copper	5.00 mg/kg	
Fluoride	1.00 mg/kg	
Iodine	0.15 mg/kg	
Iron	35.00 mg/kg	
Manganese	50.00 mg/kg	
Selenium	0.10 mg/kg	
Zinc	12.00 mg/kg	
Vitamins		
A[g]	4000.00 IU/kg	
D[g]	1000.00 IU/kg	
E[g]	30.00 IU/kg	
K_1	50.00 gm/kg	
Choline	1000.00 mg/kg	
Folic acid	1.00 mg/kg	
Niacin	20.00 mg/kg	
Pantothenate (calcium)	8.00 mg/kg	
Riboflavin	3.00 mg/kg	
Thiamin	4.00 mg/kg	
Vitamin B_6	6.00 mg/kg	
Vitamin B_{12}	50.00 μg/kg	

[a] From National Research Council (1978).

[b] Adequate to support growth, gestation, and lactation; based on 90% dry matter.

[c] Linoleic acid, 0.6%, is required.

[d] One-third to one-half can be supplied by L-cystine.

[e] One-third to one-half can be supplied by L-tyrosine.

[f] Mixture of glycine, L-alanine, and L-serine.

[g] Vitamin A, 1 IU = 0.300 μg retinol, 0.344 μg retinyl acetate, 0.550 μg retinyl palmitate. Vitamin D, 1 IU = 0.025 μg ergocalciferol. Vitamin E, 1 IU = 1 mg DL-α-tocopheryl acetate.

tail. The ears open between $2\frac{1}{2}$ and $3\frac{1}{2}$ days; incisors erupt between 8 and 10 days; and eyes open between 12 and 16 days. They are fully haired between 7 and 10 days (Baker, 1979; Farris, 1963; Lane-Petter, 1972). Maternal antibody is transferred *in utero*, via the yolk sac and by intestinal absorption of colostrum by the neonate for up to 18 days after birth (Cheville, 1976). Optimal weaning age is 20–21 days, although pups can be weaned as early as 17 days.

6. Sexing

Differentiation of sex in adult rats is relatively easy after the testes descend. The adult testes can be readily retracted through large inguinal canals. Male neonates have a larger genital papillus and the anogenital space is greater in males than females.

7. Artificial Insemination

Artificial insemination can be achieved in rats, but the major obstacle is the coagulative properties of their semen. Sperm can be obtained by maceration of the epididymis and vasa or by electroejaculation, although the latter method is unreliable and the semen often rapidly coagulates. Coagulation can be eliminated by prior surgical extirpation of the seminal vesicles and coagulating glands without significant effect on fertility. Semen can be diluted with a number of media but frozen storage of rodent semen has met with little success. Insemination can be achieved surgically by direct injection of seminal fluid into the uterus and by nonsurgical means. Successful conception seems to require not only insemination during estrus but also induction of pseudopregnancy by mating with a vasectomized male or mechanical stimulation within a few hours

(before or after) insemination. Egg harvest for transfer can be accomplished by excision of the preovulatory ovaries and teasing from gravid follicles or recovery from the oviduct or uterus by flushing with transfer medium. Superovulation by injection of gonadotropisms may enhance yield, but is usually not necessary. Eggs are generally injected directly into the uterus but the recipient uterus must be at the same stage of the uterine cycle (Bennet and Vickery, 1970).

8. Synchronization

Synchronization of estrus can be achieved by vaginal insertion of polyurethane sponges containing 0.75 mg medroxyprogesterone for 7 days. Females are then put in a cage previously occupied by male rats, sponges are removed, and the rats are injected with 3 IU of pregnant mare's serum. Within 34 hr, 93% will be in estrus. This can also be attained by administering 40 mg medroxyprogesterone in 200 ml ethanol/liter drinking water, prepared fresh daily for 6 days, then intramuscular injection of 1 IU of pregnant mare's serum (Bennet and Vickery, 1970).

E. Behavior

The rat has been utilized extensively in a variety of research fields, including behavioral science. Rats are docile, adapt to new surroundings, tend to explore, and are easily trained to a variety of sensory cues by positive or negative reinforcement. Rats sleep during daylight hours and activity, including feeding, is greater during the night and early morning. Laboratory rats are easily handled, but strain differences exist. Sprague-Dawley and LEW rats tend to be less fractious than Long Evans or F344 rats. Docility is improved with routine and proper handling. Rats become nervous and refractory to handling when they hear others squeal. Nutritional deficiency, particularly hypovitaminosis A, and mishandling can make rats vicious. Rats seek entry into small openings, a trait that is utilized for coaxing them into restrait apparatus. Like other rodents, rats are coprophagic, which must be taken into consideration when administering drugs, measuring fecal output, or performing nutritional studies.

Unlike mice, rats are less apt to fight, and males can be housed together. In addition, rats are not gregarious like mice, and seem to tolerate single caging well. Experimental studies indicate significant changes in plasma corticosteroid levels, depending on cage cohort size. Levels tend to be least in rats housed singly, to increase in groups up to 5, to decrease in larger groups up to 10–12, then rise again in groups up to 30 (Lane-Petter, 1972).

III. DISEASES

A. Infectious Diseases

Infectious agents constitute a significant environmental variable that impacts on research data derived from laboratory rats. As is the case with other species, infectious agents induce a wide range of diseases in the rat that vary from inapparent to overt clinical disease. Most investigations use large numbers of rats in which a specific group or colony consists of several to hundreds of rats. Accordingly, emphasis on disease is one of prevention and placed at the colony level rather than on a single or a few animals. Curative use of antibiotics, which is important in the treatment of bacterial diseases of nonrodent species, is rarely useful in the laboratory rat. Administration of drugs to obtain therapeutic blood levels is difficult to achieve in a colony; also some animals may improve clinically but remain colonized by the pathogen and serve as carriers, reinfecting other animals.

Rats seldom show clinical signs of disease upon arrival to the laboratory from commercial sources. However, these rats may harbor pathogens that are of low to moderate virulence and that are capable of severely compromising the health of animals when the rats are exposed to various types of experimental stress. Moreover, some of these pathogens may never cause clinical disease, yet induce microscopic lesions or biochemical aberrations that can have profound effects on research data. For these reasons, investigators and clinicians should be aware of the pathogen status of the animals used in studies, both initially and throughout the course of the studies. This section on infectious diseases contains those agents that are of principal importance to the investigative use of the rat.

1. Bacterial Diseases

a. Streptococcosis. The causative organism, *Streptococcus pneumoniae*, is a gram-positive coccus that is rather ubiquitous among humans and animals. *Streptococcus pneumoniae* is frequently recovered from respiratory tract lesions in guinea pigs, nonhuman primates, and some domestic animals. In humans, it is often present in the nasopharynx in the absence of clinical symptoms of infection. Upper respiratory tract infection of conventionally raised rats has been reported to be common. However, it is seldom present in barrier-maintained, commercial rat sources. As in pneumococcal disease in humans, a number of serological types have been associated with respiratory disease in rats.

Streptococcus pneumoniae infection in rats often remains localized in the nasopharynx without the development of overt disease. A shift in the host–parasite balance due to stress or

concurrent infection with another pathogen may result in bronchopneumonia and bacteremia. The most common signs of respiratory disease are serous to mucopurulent nasal discharge and "red tears" due to porphyrin pigments secreted from the Harderian glands, dyspnea, rales, and depressed activity. Animals will often die within a few days after the onset of pneumonic signs. The severity and prevalence of clinical disease within an infected colony are associated with environmental conditions that induce stress (e.g., experimental manipulation, overcrowding, fluctuations in ambient temperature and humidity, and copathogens). Although all age groups are susceptible to infection and clinical disease, young animals are more apt to be clinically affected. Transmission between rats is by aerosol droplet. Although both humans and rats can carry the same serotypes of *S. pneumoniae,* the authors are unaware of evidence indicating zoonotic or human-to-animal transmission.

The most characteristic gross lesions are pulmonary consolidation and fibrinopurulent pleuritis and pericarditis (Fig. 1). An extensive fibrinopurulent peritonitis, orchitis, or meningitis may occur as well. If a bacteremia occurs early, the disease may be acute with few gross lesions. *Streptococcus pneumoniae* induces an outpouring of exudate rich in fibrin, neutrophilic leukocytes, and erythrocytes into the alveoli. Bronchioles are filled with neutrophilic leukocytes. Embolic lesions may occur in multiple tissues which include the spleen, liver, kidneys, joints, and brain.

Streptococcosis is diagnosed by clinical signs, characteristic lesions, and isolation of *S. pneumoniae* from lesions. The pericarditis, pleuritis, and pleural effusion noted above differentiate pneumococcal disease from pneumonia due to *Mycoplasma,* although the two pathogens often are superimposed. This organism produces an α-hemolysis on blood agar plates similar to that of the *Streptococcus viridans* group. *Streptococcus pneumoniae* isolates are most commonly differentiated from nonpathogenic *S. viridans* by the sensitivity of the former organism to Optochin (hydrocuprein hydrochloride). Optochin-impregnated discs are placed on a blood agar plate which has been inoculated with a pure culture of the clinical isolate. If the isolate is *S. pneumoniae,* a distinct zone of growth inhibition will be present around the disc. Although typing of *S. pneumoniae* isolates is seldom done today, one can type an isolate by reacting known specific *S. pneumoniae* antisera with *S. pneumoniae* isolates. This serological test is the Neufeld–Quellung reaction and is based on the capsular swelling that is induced by specific antiserum.

There is no effective means to control *S. pneumoniae* infection once it is enzootic in a colony. Benzathine penicillin (30,000 units/200 gm body weight) may be helpful in reducing the severity of the disease and as an aid in limiting infections to a subclinical mode in some animals. However, antibiotics will not eliminate the organism from rat colonies. Hysterectomy rederivation of breeding stock from infected colonies is an effective method of initiating new stock free from pneumococcal infection (Weisbroth, 1979).

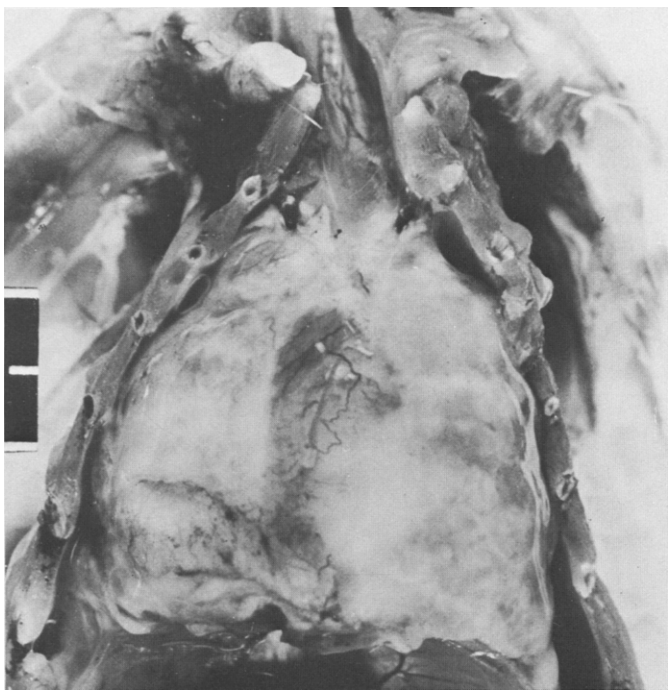

Fig. 1. Streptococcus pneumoniae infection. Thoracic viscera are covered with fibrinopurulent exudate.

b. Pseudotuberculosis (Corynebacteriosis). The causative agent of pseudotuberculosis is the gram-positive bacillus, *Corynebacterium kutscheri.* On occasion, other *Corynebacterium* species can cause similar syndromes in rats. Typically, the organism causes inapparent infections in rats, with exacerbation of respiratory disease under conditions of stress. When clinically ill, the most commonly seen signs include serous oculonasal discharge, dyspnea, anorexia, and loss of weight or retarded growth. Animals with severe pulmonary signs usually succumb within several weeks, while rats with less severe signs often survive much longer. Most rats will have inapparent infections in which *C. kutscheri* cannot be isolated from internal organs. Little is known concerning how *C. kutscheri* is carried or transmitted within a colony. It has been suggested that the organism is transmitted via aerosol droplet or direct contact. Once rats are infected, a hematogenous spread may be involved, since lung lesions are initially interstitial and not bronchial.

Gross lesions are characterized by a variable number of grayish-yellow foci surrounded by red zones, particularly in the lung (Fig. 2). In longer-standing cases, individual foci coalesce into raised lesions 1 cm or larger in diameter. Occasionally, fibrous adhesions occur between the lungs and thoracic walls. Similar lesions may be seen in other organs, including

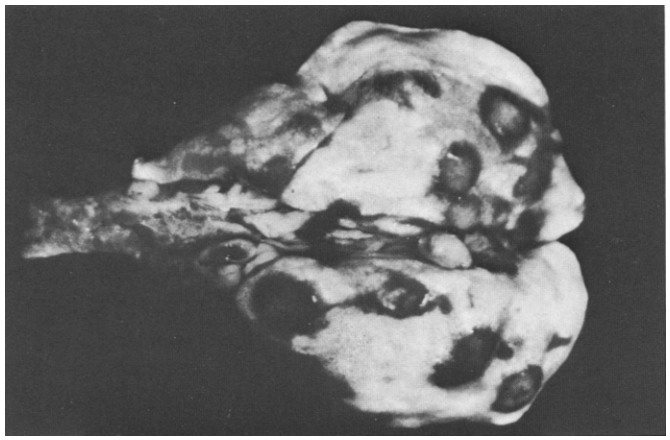

Fig. 2. Corynebacterium kutscheri infection. Lungs contain multiple pale nodular foci surrounded by zones of hyperemia.

the liver, brain, and kidneys. The hepatic lesions resemble tubercles and have caseous centers and fibrous capsules. Prepucial adenitis, arthritis, and otitis media may also be caused by *C. kutscheri*.

The lesions in various target organs appear to be due to septic emboli. Pulmonary lesions initially consist of a polymorphonuclear cell and macrophage infiltrate of the bronchioles and interstitial tissue with a round cell infiltrate occurring later. Bronchi become impacted with polymorphonuclear cells and necrotic leukocytes. Giemsa or Gram staining of infected tissues will reveal the rod-shaped *C. kutscheri* organisms.

Diagnosis of *C. kutscheri* infection is made on clinical signs, gross and microscopic lesions, and isolation of the bacterium from infected tissues. Although the respiratory signs are similar to those present with mycoplasmosis, the rapidity with which *C. kutscheri* clinically affected rats succumb helps differentiate it from *Mycoplasma pulmonis*-induced disease. Unlike streptococcosis, fibrinopurulent pericarditis, peritonitis, and pleural effusion are not seen. Whereas peribronchial lymphoid hyperplasia is a dominant lesion in mycoplasmosis, it is unremarkable in *C. kutscheri* infections. *Corynebacterium kutscheri* is easily recovered from lesions and upper respiratory tract exudates by culturing on blood agar plates incubated aerobically at 37°C.

Epizootics of pseudotuberculosis may occur in conventionally raised breeding colonies, but rarely occur in barrier-raised colonies. Epizootics often can be retrospectively associated with an environmental stress (e.g., fluctuation in ambient temperature or ventilation). Culling of ill animals will not eliminate *C. kutscheri* from animals remaining in a colony. Isolation of the organism from animals with subclinical infections is not usually successful. For this reason, cortisone administration has been advocated as a means for surveillance of infection in colonies prior to necropsy and culturing for *C. kutscheri*. In the past, most serological methods have been unsatisfactory in detecting antibody in animals with inapparent infections (Weisbroth, 1979). Recently, however, enzyme-linked immunoabsorbant assay (ELISA) has been shown to be capable of detecting antibody in animals without clinical signs of infection (Ackerman *et al.*, 1984). Hysterectomy derivation is an effective means to establish a *C. kutscheri*-free colony. Antibiotic therapy will not eliminate *C. kutscheri* from a colony, but a 7-day regimen of penicillin has been reported to be effective in curtailing an epidemic of *C. kutscheri*-induced pneumonia (Fox *et al.*, 1979).

Since *C. kutscheri* infection is, in most cases, inapparent and manifests itself whenever the host is sufficiently stressed, it can be a significant problem in experimentally stressed rats.

c. Tyzzer's Disease. Tyzzer's disease is caused by the gram-negative, spore-forming rod, *Bacillus piliformis*. This organism, which is not a true *Bacillus*, is an intracellular pathogen that has not been cultivated on artificial media, and is, as yet, taxonomically undefined. In the laboratory, *B. piliformis* is propagated in the yolk sac of embryonated chick eggs.

This disease occurs in other rodent species and appears to be widely distributed in many nonrodent species, but there appears to be a degree of species specificity among *B. piliformis* strains. It occurs occasionally in conventionally raised rat colonies. The vegetative form of *B. piliformis* is unstable in the environment. However, spores of the organism are relatively stable and are believed to be the source of transmission among animals.

Clinical signs associated with Tyzzer's disease are not particularly distinctive and, accordingly, only suggestive in making a diagnosis. Typically, affected rats are apt to be adolescents with signs such as lethargy, weight loss, and distended abdomens. Diarrhea is not a common sign in rats with *B. piliformis* infection. Animals displaying clinical signs generally die within several weeks. Clinically inapparent infections occur and are most probably responsible for transmission of the organism within a colony. Clinically evident Tyzzer's disease is usually associated with experimentation that compromises the immunocompetence of rats.

The most remarkable gross lesions involve the liver, ileum, and myocardium. Hepatic lesions consist of numerous small, pale foci on the surface and within the parenchyma. The intestinal lesion has been termed "megaloileitis" due to a segmental dilatation and inflammation of the ileum (Fig. 3) (Jonas *et al.*, 1970). Ileal distension is not always present. In some rats, circumscribed gray foci also occur in the myocardium.

The pathogenesis of the disease is believed to involve a primary intestinal infection with spread to the liver via the portal circulation. *Bacillus piliformis* invades enterocytes, resulting in villus shortening, inflammation, necrosis, and hemorrhage. Intracellular organisms are demonstratable in epithelium of

Fig. 3. *Bacillus piliformis* infection (Tyzzer's disease). The distal small intestine is dilated, congested, and hemorrhagic; also referred to as megaloileitis. (From A. M. Jonas, D. H. Percy, and J. Craft. Tyzzer's disease in the laboratory rat. *Archives of Pathology* **90,** 516–528. Copyright 1970, American Medical Association.)

crypts and villi. The necrotic foci in the liver are most often present near vessels. Surrounding these foci are varying numbers of leukocytes, macrophages, and fibroblasts. Intracytoplasmic bacteria may be seen in hepatocytes at the periphery of the lesions, but may be present in very small numbers and thus be hard to find. Organisms are also found in myocardium around foci of necrosis (Weisbroth, 1979).

A presumptive diagnosis can be made by the gross lesions, but a definitive diagnosis is dependent upon observation of the organism within hepatocytes, intestinal epithelium, or myocardium. Impression smears of liver taken at necropsy and stained with Gram, Giemsa, or methylene blue stains may be useful for a rapid diagnosis. However, formalin-fixed specimens stained by Giemsa or Warthin–Starry methods are usually performed to confirm a diagnosis. The ileal distension seen in rat Tyzzer's disease must be differentrated from other causes of adynamic ileus, particularly chloral hydrate-induced lesions.

Prevention of Tyzzer's disease in a colony is dependent upon a barrier that excludes entry of the agent by contaminated cages, equipment, and infected animals. Routine cage sanitation probably is ineffective in killing the spores of *B. piliformis,* but exposure of spores to 80°C for 30 min has been shown to inactivate them. Sodium hypochlorite (0.3%) is an effective disinfectant (Ganaway, 1980). Although antibiotics have been shown to be effective under experimental conditions in mice, there is no evidence to indicate that antibiotic therapy can be of value under natural conditions within a colony of rats (Weisbroth, 1979).

d. Pasteurellosis. *Pasteurella pneumotropica* frequently infects conventionally raised rats and has been recovered occasionally in rats from barrier- and axenic-maintained colonies. It is a pathogen of very low virulence, and most infections remain clinically inapparent. Only a relatively few reports document *P. pneumotropica* as a primary pathogen in cases of penumonia, otitis media, and conjunctivitis. As a copathogen with either *Mycoplasma pulmonis* or Sendai virus, it has a contributory role in the resultant respiratory lesions and otitis.

Its localization is not limited to the respiratory tract, since it is frequently isolated from the oral cavity, intestinal tract, and uterus. It also has been associated with mastitis and furunculosis in rats. It has been suggested that *P. pneumotropica* is essentially an enterotropic rather than a pneumotropic organism. The intestinal tract is probably the primary site for localization of the organism in subclinical infections.

Horizontal transmission is by the oral–fecal route and direct contact. Since *P. pneumotropica* is frequently carried in the uterus, vertical transmission can occur, and, accordingly, this can compromise the microbial status of axenic and gnotobiotic colonies.

Distinctive clinical signs and lesions do not occur with *P. pneumotropica*-induced disease. Accordingly, a diagnosis must be based upon its isolation as the sole pathogen or, as in many cases, as a copathogen within lesions. Blood agar medium is satisfactory for primary isolation from nonenteric sites. However, for recovery from the intestinal tract, enrichment in a medium such as GN broth is recommended before isolation is attempted on blood agar plates (Weisbroth, 1979).

Hysterectomy derivation and barrier maintenance are the only means to control infection. However, particular attention must be made to ensure that hysterectomy-derived young came from dams that had culturally negative uteri. Antibiotic therapy is not effective in eliminating the organism from a colony.

e. Salmonellosis. *Salmonella* species that infect rats include *Salmonella enteritidis, S. typhimurium, S. dublin,* and *S. meleagridis.* Salmonellosis, which was once a major cause of disease in laboratory rat and mouse colonies, is rarely reported in either species today. However, it still exists in wild populations of rodents and, therefore, remains a potential threat to laboratory rodents.

Infection in an immunologically naive colony typically results in an epizootic of clinically affected rats and a varying proportion of animals with inapparent infection. These latter animals act as subclinical carriers to render the infection as enzootic in a colony. Acute outbreaks will occur intermittently whenever immunological and other host defense mechanisms

are altered. Signs associated with salmonellosis in the rat are anorexia, depressed activity, starry hair coats, and soft to formless feces. Affected animals die in 1 to 2 weeks.

Lesions that occur in salmonellosis differ depending on the stage of the disease. Salmonellae penetrate the intestinal mucosa at the level of the ileum and cecum. The earliest lesions occur in this locale and consist of a mild dilatation, thickened intestinal walls, and a granular mucosal surface. Involvement of the reticuloendothelial system is reflected by enlarged Peyer's patches, mesenteric lymph nodes, and spleen. In some infected animals, a bacteremic state occurs that results in the demise of the host before the development of further lesions. However, in animals not succumbing to septicemia, ulceration of the ileal, colonic, and cecal mucosa occurs. Histologically, the villus epithelium of the ileum is markedly degenerated, and the lamina propria is infiltrated with neutrophils and macrophages. Concomitant with intestinal lesions is the development of focal necrosis and granulomas in the spleen and liver due to hematogenous spread of the organism (Buchbinder *et al.*, 1935; Maenza *et al.*, 1970).

In rats who are intermittent or chronic shedders of salmonella, the most remarkable lesions are lymphadenitis of the mesenteric lymph nodes and ulceration of the cecal mucosa. Rats from which salmonella is chronically shed have more advanced lesions than do intermittent shedders of the organism.

A diagnosis of salmonellosis relies upon identification of an isolate as a *Salmonella* sp. Recovery of salmonella from the intestines, spleen, and liver is readily accomplished in rats clinically affected during an epizootic. However, this is not true for asymptomatic carriers, since some will shed the organism intermittently in the feces, and recovery from tissues is difficult. Recovery in carrier animals is best accomplished by initial incubation of fecal pellets in an enrichment broth, such as selenite F plus cystine broth, followed by streaking onto brilliant green agar (Weisbroth, 1979). From this medium, possible salmonella colonies are inoculated into triple-sugar-iron slants. Final identification is then made by biochemical tests and serotyping.

Prevention of this disease is based upon the exclusion of wild rodents from laboratory animal facilities and the use of only feed and bedding that has been properly processed and packaged to ensure against salmonella contamination.

f. Pseudomoniasis. *Pseudomonas aeruginosa*, a ubiquitous gram-negative bacterium found in soil and water, colonizes plants, insects, animals, and humans. It often colonizes the oropharynx and can be isolated from the intestinal tract of rodents. Infection with this organism in immunocompetent rats is nearly always inapparent. However, when rats are immunosuppressed, *P. aeruginosa* invades the upper respiratory mucosa and cervical lymph nodes, becomes bacteremic and induces an acute, lethal disease. In some cases, rats develop facial edema, conjunctivitis, and nasal discharge. In genetically thymic-deficient rats (nude), retro-orbital abscesses may occur prior to bacteremia.

Transmission in laboratory rodents occurs primarily by direct contact and contaminated water bottles and automatic watering systems. Phenolics are usually effective disinfectants, but quaternary ammonium compounds may actually support its growth.

Diagnosis of pseudomoniasis is based upon a history of immunosuppression associated with an epizootic of acute disease and isolation of *P. aeruginosa* from the blood and organs of affected rats. Facial edema in affected rats must be differentiated from viral sialodacryoadenitis.

Pseudomonas aeruginosa grows well on blood agar and most other standard laboratory media. Most strains are β-hemolytic and produce a bluish-green pigment, pyocyanin, as well as fluorescein. The use of specialized media (Pseudomonas P agar) enhances pigment production. The organism derives energy from carbohydrates via oxidation rather than fermentative metabolism. Identification of isolates as *P. aeruginosa* is easily made by the above characteristics and appropriate biochemical reactions (Weisbroth, 1979).

In most research applications, *P. aeruginosa*-free rats are not necessary for the conduct of the work. It is a major problem, however, in rats used for burn research and in studies in which drugs or radiation induce immunosuppression. Infection can be relatively well controlled in a colony by hyperchlorinating drinking water at 12 ppm or by acidification of water to a pH of 2.5–2.8. In a closed colony, it is also advisable to remove rats that remain culturally positive after water treatment has been instituted. In studies requiring pseudomonas-free rats, isolators are useful in which a gnotobiotic environment can be achieved. Alternatively, laminar flow units may suffice if supplies and equipment are sterilized and personnel wear sterile garments.

g. Streptobacillosis. *Streptobacillus moniliformis* is a commensal bacterium often present in the nasopharynx of conventionally raised rats. Although it may be involved occasionally as a secondary invader within inflammatory lesions of the rat, the chief importance of *S. moniliformis* is that it is the principal agent causing rat-bite fever in humans (Anderson *et al.*, 1983). The other bacterium associated with this clinical syndrome is *Spirillum minus*. Clinical signs in humans usually occur within 10 days of a rat bite and consist of headache, weakness, fever, a generalized rash, and arthritis. Often clinical signs subside in several days but then recur at irregular intervals for weeks or months (see Chapter 22).

2. Mycoplasmal Diseases

a. Murine Respiratory Mycoplasmosis. Murine respiratory mycoplasmosis (MRM) is the term now accepted for a disease which, for many years, had an undefined etiology and a

number of synonyms [i.e., infectious catarrh, enzootic bronchiectasis, chronic respiratory disease (CRD), and chronic murine pneumonia]. Since 1969, the causal relationship of *Mycoplasma pulmonis* with this disease has become well established (Kohn and Kirk, 1969; Lindsey *et al.*, 1971; Whittlestone *et al.*, 1972). Of all the pathogens occurring in laboratory rats, *M. pulmonis* has had the greatest negative impact on studies. This has been primarily due to the chronicity of the disease, which often manifests itself only after months of infection. Long-term studies in areas of toxicology, carcinogenesis, nutrition, and gerontology, in particular, have been affected. Prior to the use of gnotobiotic techniques and barrier maintenance in rat production colonies, *M. pulmonis* was enzootic in nearly all commercial and institutional colonies. Today, vendors can be selected who offer mycoplasma-free rats. *Mycoplasma pulmonis* is highly contagious and induces a disease that frequently results in debilitation or demise of the host after a long period of time.

The clinical signs associated with MRM range from negligible upper respiratory tract signs to systemic signs associated with pneumonia. The earliest and most common signs include snuffling and serous or mucopurulent oculonasal discharge. Extension of *M. pulmonis* infection from the nasopharynx via the eustachian tubes to the middle ears is common. However, torticollis and circling due to involvement of the inner ear are infrequently observed, even though one or both middle ear bullae may be impacted with exudate. The onset of upper respiratory signs is variable, but often occurs within several weeks postinfection. Signs of penumonia include dyspnea, rales, and systemic effects such as weight loss, starry hair coat, and hunched posture. Characteristically, signs of pneumonia occur 3–6 months postinfection, but this is quite variable and is a function of environmental influences, such as intracage ammonia levels and the immune competence of the host. In a small percentage of cases, the disease will be nearly subclinical even in the presence of extensive pulmonary lesions.

Mycoplasma pulmonis is transmitted both horizontally and vertically from dams to their litters. In most instances, transmission from the female occurs postpartum by direct contact, but if the genital tract of the dam is infected, antenatal infection can occur. Horizontal transmission between postweanling rats of any age readily occurs, and there appears to be no significant age-related resistance to either infection or disease. Although little is known about differences in resistance among rat stocks and strains, the LEW rat has been shown to be more susceptible to MRM than the F344 rat. There is little evidence available to indicate that transmission occurs through fomites such as caging equipment and garments worn by personnel. Since aerosol droplet and direct contact appear to be the primary modes by which *M. pulmonis* infections are spread, the rapidity with which the organism is transmitted is dependent upon environmental factors, such as ventilation rates, degree of recirculation of air, and animal density within rooms.

The basis for the pathogenicity of *M. pulmonis* is not well understood. *Mycoplasma pulmonis* adsorbs to the cell membrane of the ciliated, columnar or cuboidal epithelia in the respiratory tract (Fig. 4). It has been suggested that adsorption is a means by which mycoplasmas damage host cells by uptake of essential cellular metabolites; release of cytotoxic products, such as H_2O_2; or cross reaction of antibody with cell membrane components that are antigenically similar to or altered by mycoplasmas. Infection severely distorts or ablates ciliary structures (Fig. 4), interfering with mucociliary clearance mechanisms.

The gross lesions in the upper respiratory tract include mucopurulent exudate in the nasal cavity, sinuses, and middle ear bullae. Later, the exudate becomes caseous within the bullae. Lesions in the lower respiratory tract reflect those of a bronchopneumonia. The earliest lesion is a mucopurulent exudate within the trachea, bronchi, and bronchioles. This precedes grossly evident lesions of the lung parenchyma that initially consist of atelectasis due to bronchial occlusion. Later, bronchiectatic lesions appear as numerous cream-colored nodular abscesses on the surface of the lung. These lesions may be restricted to only a portion of a lobe or may involve nearly all of the parenchyma (Fig. 5).

Microscopically, the inflammatory response is characterized by a lymphocyte and plasma cell infiltrate in the submucosa and neutrophilic leukocyte response within the lumina of the

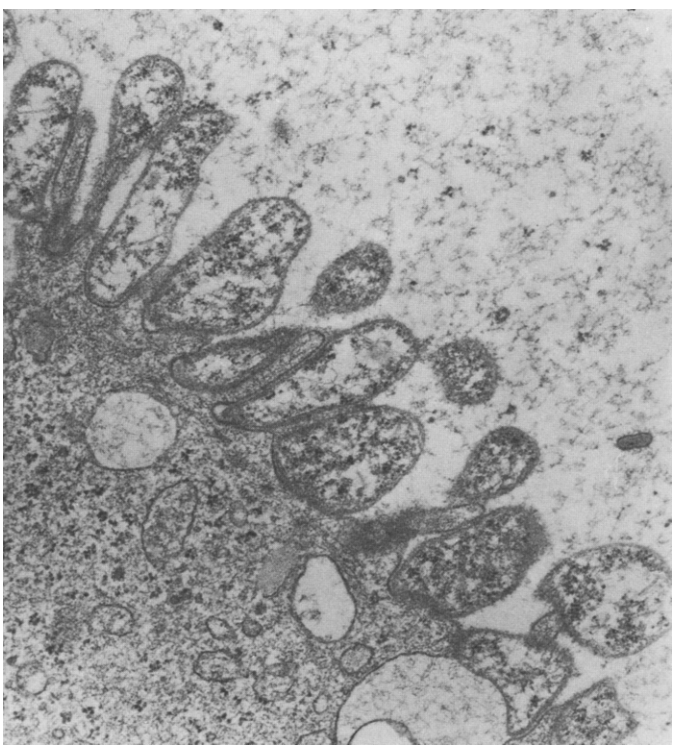

Fig. 4. Electron micrograph of *Mycoplasma pulmonis* attached to tracheal epithelium.

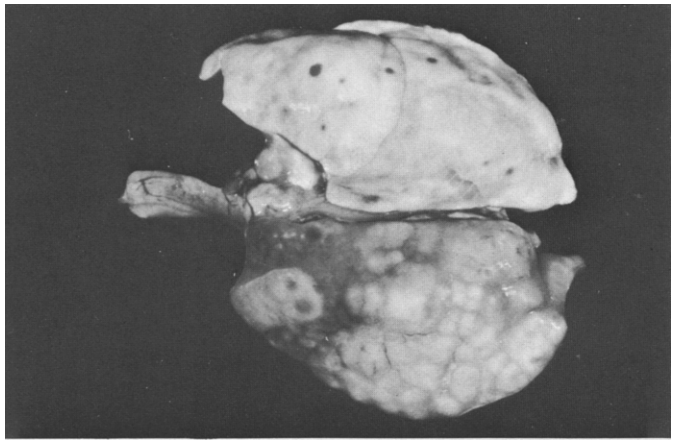

Fig. 5. Mycoplasma pulmonis infection (murine respiratory mycoplasmosis). Lungs are unevenly consolidated, and the pleural surface is elevated due to bronchiolectasis and abscess formation.

nasal cavity, eustachian tubes, middle ears, and tracheobronchial tree. A consistent and prominent lesion in the lung is the peribronchial lymphoid hyperplasia that often becomes quite massive. Within the lumina of the bronchi and bronchioles, mucin and neutrophil exudation increases during the course of the disease to the point of bronchiectasis. Concomitant with the impaction of bronchi is a change in the epithelia from a ciliated, columnar type to a squamoid type. This change in epithelial architecture is likely associated with cytotoxic enzymes from autolyzed neutrophils, although a direct cytotoxic effect from mycoplasmas could be involved.

A tentative diagnosis of MRM can usually be made by observance of the clinical signs and gross lesions described above. Clinical signs alone are not particularly helpful, since nasal exudates are present in bacterial infections such as *S. pneumoniae*. In addition, the reddish porphyrin deposition seen in the nares and periorbitally in sialodacryoadenitis virus infection and water deprivation may be confused with exudation. The gross lesions of otitis media and bronchiectasis are rather distinct. However, *C. kutscheri* lung lesions may grossly mimic those of MRM. Histopathology and serological evidence will differentiate MRM from Sendai virus infection, although the two infections are often superimposed. Recently a filamentous bacterium has been associated with bronchiectasis in wild and laboratory rats (MacKenzie *et al.*, 1981). However, the causal relationship of this organism with lesions is undefined since the rats were also infected with *M. pulmonis*. This filamentous bacterium has not been successfully grown on artificial media, and its presence is best verified by either histology, using the Warthin-Starry Stain, or electron microscopy (Fig. 6). Although a definitive diagnosis of MRM is made by isolation of *M. pulmonis* from involved tissues, it is evident that the existence of other agents must be evaluated to determine if copathogens are contributory to lesions.

Prevention of MRM in either breeding or experimental colonies is dependent upon barrier systems that preclude the entry of *M. pulmonis* into the facility. Hysterectomy derivation is the only means of establishing an *M. pulmonis*-free breeding colony from a previously infected stock. Due to the frequent localization of this microorganism in the uterus, it is necessary to ensure that neonates taken by hysterectomy have not been infected *in utero*. Rats used in research animal facilities are obtained from various commercial and institutional sources. Accordingly, it is essential that the mycoplasma status of these sources is known and that the rats are housed by vendor or in groups with a similar microbial status.

For assessment of whether a group of rats is *M. pulmonis*-free, the best sites for isolation in animals without gross lesions are the nasal cavity, middle ear, trachea, and uterus–oviduct. *Mycoplasma pulmonis* is not particularly fastidious and grows well in several types of mycoplasma media (Cassell *et al.*, 1979; Lentsch *et al.*, 1979). Most formulations have a pH indicator that is useful since *M. pulmonis* ferments glucose. In broth media, moderate to heavy growth is reflected by pH and color of the broth. In broth cultures in which the titer is low, a perceptable pH change may not occur. Tissue and washing samples should be placed in broth rather than agar media, since recovery of the organism is more likely in those samples containing few mycoplasmas. Samples from broth cultures are transferred to agar media when a pH change is readily evident or at 7–10 days if no pH change occurs. Mycoplasma colonies are evident in 3–4 days by observation with 40× stereoscopic microscopy.

Although culturing and histopathology have been the usual means to survey rat colonies, ELISA testing has recently been shown to be a very sensitive serological assay and one that can be performed quickly in most clinical laboratories (Cassell *et al.*, 1981a). *In vitro* sensitivity tests show *M. pulmonis* to be susceptible to tetracycline and tylosin. Tetracyline, given at 5 mg/ml drinking water, may be useful in some situations (Lindsey *et al.*, 1971). However, treatment with antibiotics seldom influences the disease course of MRM in a colony situation.

b. Murine Genital Mycoplasmosis. *Mycoplasma pulmonis* recently has become recognized as an important pathogen in the female genital tract of rats, and thus is being treated here as a distinct disease rather than as a sequella to MRM. Infection of the genital tract is usually inapparent. However, reduced fertility and fetal deaths can occur. Infection of the oviduct and uterus occurs frequently in rats who have respiratory mycoplasmosis. It is unknown whether localization in the genital tract occurs due to a hematogenous spread or to an ascending infection of the genital tract. It has been shown that subsequent to intravenous inoculation, *M. pulmonis* almost invariably localizes in the female oviduct–uterus.

Gross lesions, when present, consist of a purulent oophoritis, salpingitis (Fig. 7), and pyometra. The LEW strain is particu-

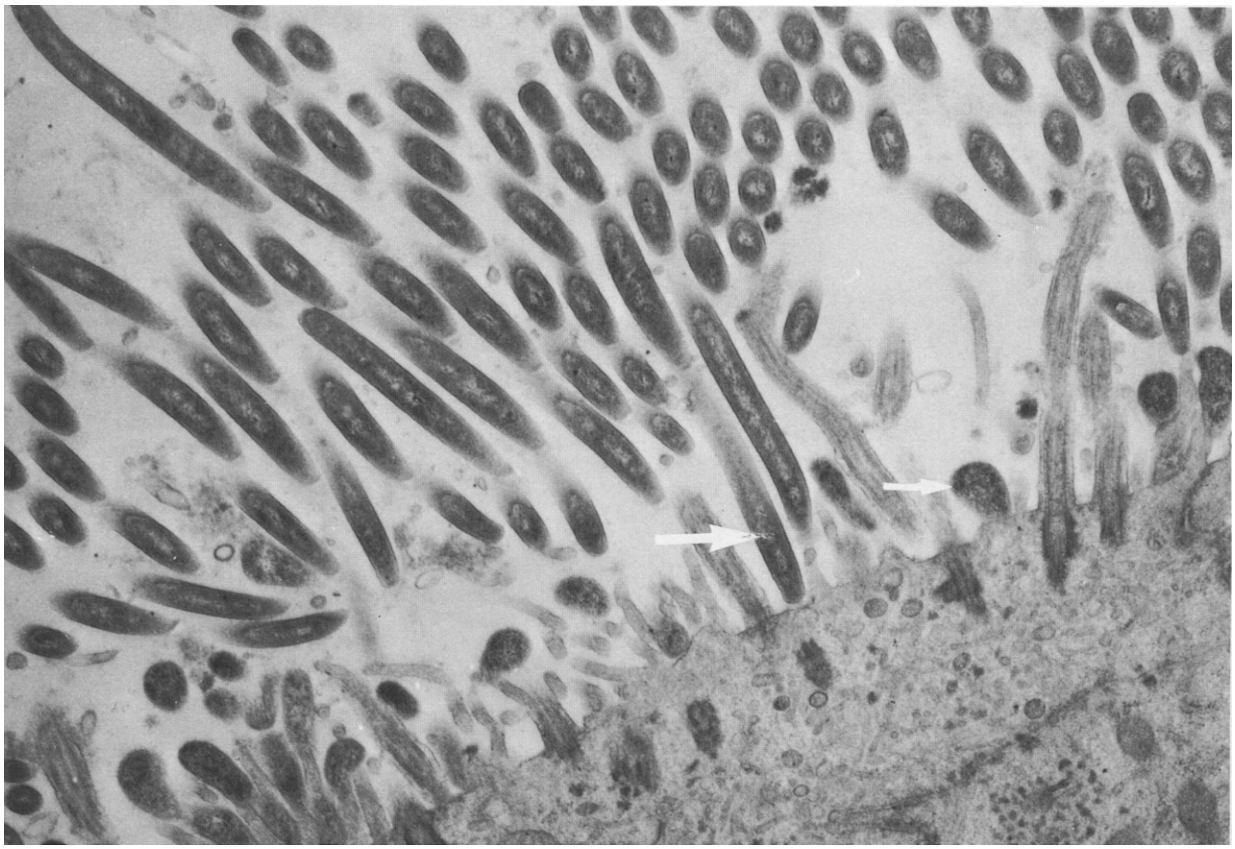

Fig. 6. Electron micrograph of filamentous bacterium (large arrow) and *M. pulmonis* (small arrow) attached to epithelium of respiratory mucosa. The morphology of size of the filamentous bacterium are similar to that of the cilia. (Courtesy of Dr. W. F. MacKenzie.)

larly prone to develop gross lesions. *Mycoplasma pulmonis* adsorbs to the epithelial cells in the genital tract in a manner similar to that seen in the respiratory tract. Salpingitis occurs most frequently and is characterized by exudation of neutrophils into the lumen, hyperplasia of oviductal epithelium, and a lymphoid response in the submucosa. The lesions in the ovarian bursa include edema and inflammation. Uterine lesions can vary from a mild inflammatory change to pyometra (Cassell *et al.*, 1981b).

Genital mycoplasmosis in the male rat has not been well documented. However, it is known that experimental inoculation can include an inflammatory response in the ductus efferens and epididymis. Moreover, it is known that *M. pulmonis* is capable of adherence to spermatozoa in an *in vitro* system.

Since *Pasteurella pneumotropica* can also induce similar lesions in the female rat, a diagnosis of mycoplasmosis is dependent upon isolation of *M. pulmonis* from the lesions. Methods for culturing and identification are similar to those used for respiratory mycoplasmosis.

Because the rat is widely used in various types of reproductive biology research, *M. pulmonis* colonization, even without gross lesions, would probably impact on the validity of data. The grossly evident caseous lesions in the ovary and oviduct can be mistaken for neoplasia if microscopy is not done.

c. Mycoplasmal Arthritis. The etiological agent of this disease is *Mycoplasma arthritidis*. This mycoplasma species colonizes the pharynx, middle ears, and lungs of rats, although few studies have been done to document the relative frequency of this mycoplasma in rat sources. Within the respiratory tract, *M. arthritidis* colonization is thought to induce negligible lesions, and it has been shown to coexist with *M. pulmonis*.

Although it is often considered to be the principal agent involved in arthritis in rats, the disease has been rarely reported. Nearly all reports of its involvement in clinically apparent arthritis have been made prior to 1960. It has been suggested that poor cage sanitation and abrasions of the extremities are involved in entry of the organism to the joints by hematogenous spread or extension from surrounding tissues (Ward and Cole, 1970). Since the organism appears to be of low virulence, the immunocompetence of the host may be a major factor in the outcome of infection.

Arthritic animals limp and move with difficulty due to pain associated with the polyarthritis. Any of the joints in the limbs and vertebrae can be affected, but the tibiotarsal and radiocarpal joints are most often involved. Affected joints are hyperemic and swollen. Incised joints reveal a purulent exudate in both articular and periarticular tissues. Microscopically, there is exudation of neutrophils into the synovial spaces, and a lymphocyte and plasma cell infiltration in the synovial membranes. Destruction of the articular cartilage occurs subsequent to the inflammatory response.

Since polyarthritis can occur subsequent to septicemias associated with other bacteria, particularly *C. kutscheri*, a diagnosis of *M. arthritidis*-induced arthritis is contingent upon the demonstration of *M. arthritidis* by isolation or immunofluorescence techniques. This mycoplasma species grows well in media used to isolate *M. pulmonis* if arginine is added to the formulation (Cassell *et al.*, 1979).

Tetracyclines have been used to prevent the onset of arthritis when the organism has been inoculated intravenously, but there are no reports of its efficacy in spontaneous cases. *Mycoplasma arthritidis*, like *M. pulmonis*, may contaminate transmissible tumors and caution should be exercised to ensure transplanted tissues are not contaminated.

3. Rickettsial Diseases

Hemobartonellosis. The causative agent of this rickettsial disease is *Hemobartonella muris*. This organism is an extracellular parasite of erythrocytes and induces inapparent infections that may persist for long periods. The ability of the host to restrict the infection to a subclinical mode rests with the integrity of the reticuloendothelial system. Evidence of infection is usually limited to splenomegaly and laboratory findings of mild parasitemia and reticulocytosis.

Transmission of *H. muris* involves the blood-sucking louse, *Polyplax spinulosa*. Transmission can occur during a blood meal or when rats crush infected lice and are inoculated via pruritis-induced abrasions. The organism can also be transmitted inadvertantly with transplantable tumors and other biological products.

Diagnosis of hemobartonellosis is dependent upon identification of the organism in the peripheral blood of infected animals. The usual method of detection is by splenectomizing rats suspected of harboring the organism. In these rats, severe parasitemia and hemolytic anemia occur within 2 weeks after surgery. *Hemobartonella muris* can be visualized on the surface of erythrocytes in Romanowsky-stained blood smears as coc-

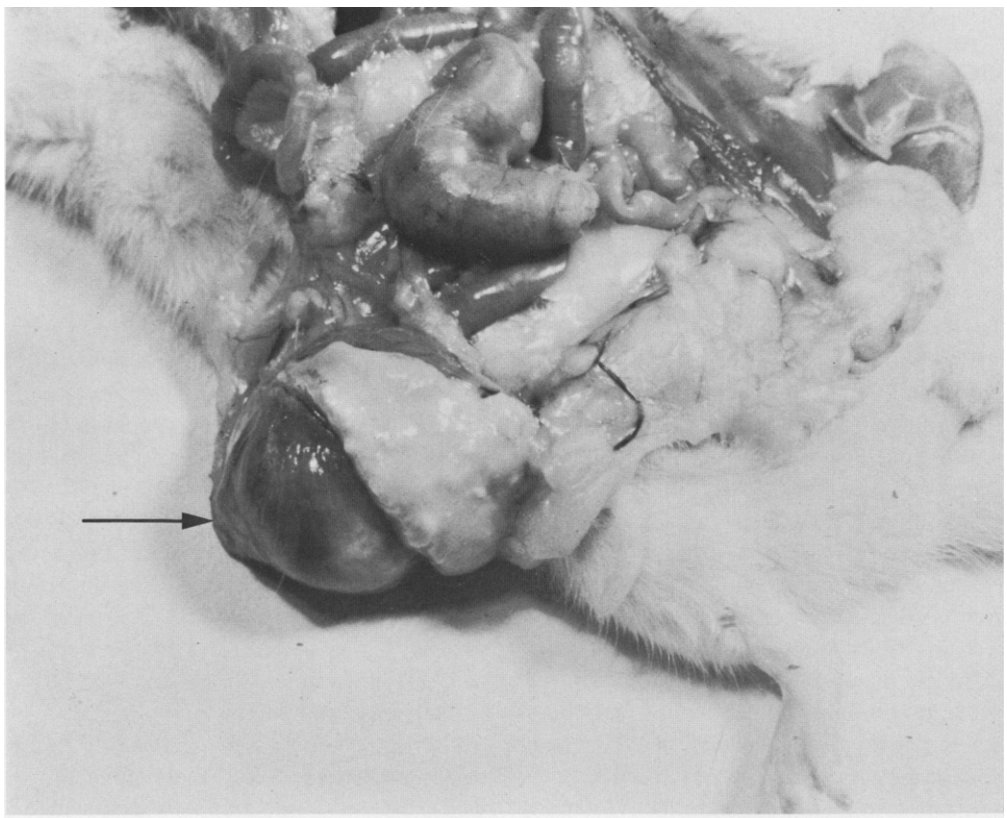

Fig. 7. Genital *Mycoplasma pulmonis* infection. Uterine wall (arrow) has been excised to show exudation within lumen of uterus.

coid bodies arranged singly, in clusters, or chains (Cassell *et al.*, 1979).

The rarity of reported cases would indicate *H. muris* is no longer a significant problem in barrier-maintained colonies. However, conventionally maintained colonies may be exposed to infected wild rats and *P. spinulosa* and, accordingly, the disease still is of importance in the laboratory rat. The disease has had a negative impact on investigations of various types, but principally with those in which the host's immune competence has been impaired.

4. Viral Diseases

a. Parvoviral Syndromes. Parvoviruses that can infect rats include rat virus (RV), Toolan H-1 (H-1) virus, and minute virus of mice (MVM). Parvoviruses are small nonenveloped viruses that resist extremes in temperature, pH, and drying. Rat virus, or Kilham rat virus (KRV), has several antigenically related strains (RV, H-3, X-14, L5, HB, SpRV, HER, HHP, Kirk), all of which have been isolated as inadvertant contaminants of rat tissue or rat-passaged biological material. Toolan H-1 related serotypes (H-1 and H-T) are antigenically distinct from RV serotypes. Both RV and H-1 are experimentally pathogenic, producing similar lesions, but only RV has been associated with natural disease. Neonatal rats can be experimentally infected with MVM, but the virus does not seem to cause natural infection. Minute virus of mice antibody reactivity can be present in rat serum, but this is probably nonspecific, since it can be found in germfree rat serum and is reduced or eliminated by receptor destroying enzyme.

Rat virus infection is usually subclinical or latent, but a number of clinical syndromes have been associated with it. Infection of pregnant females can cause fetal resorption and birth of small litters. Pups are runted, atactic, or jaundiced. Neonates develop similar signs following postpartem exposure. Rats introduced to an infected colony can develop ruffled fur, dehydration, and sudden high mortality. A similar syndrome occurs in latently infected adults subjected to immunosuppressive regimens.

The rat is the only natural host for RV and H-1, although experimental infection can be established in a number of other species. Seroconversion to both RV and H-1 virus is common, with a high prevalence of infection within an enzootically infected colony. Horizontal transmission is achieved by the oral and probably respiratory routes, with virus excretion primarily in the feces. Some strains of RV can be excreted in the milk or *in utero*. Clinical signs are manifest transiently upon introduction of RV into a previously uninfected population, but, thereafter, the virus spreads rapidly to produce subclinical or inapparent enzootic infection. Rat virus can persist as a true latent infection in the presence of high circulating antibody, but disease can be activated by immunosuppression. It must, therefore, be assumed that seropositive rats are persistently infected and can serve as a source of infection to other rats.

Pups infected *in utero* or as neonates develop intranuclear inclusions and necrosis in the outer germinal cell layer of the cerebellum. The recovered animal has severe depletion of the internal granular layer and disorganized Purkinje cells. Intranuclear inclusions are also in hepatocytes, Kupffer cells, endothelial cells, and biliary epithelial cells, resulting in necrotizing hepatitis and the sequellae thereof (bile retention, jaundice, peleosis, bile ductal hyperplasia, parenchymal collapse, nodular hyperplasia). In adults, infection is usually inapparent, but when acute disease is precipitated, RV injures vascular walls and hematopoietic elements, causing coagulative disorders, thrombosis, hemorrhage, and infarction within the central nervous system (hemorrhagic encephalomyelopathy). Hemorrhagic and necrotic lesions have also been noted in the peritoneum, testis, and epididymis. Rat virus has broad tissue tropism and lesions or clinical signs may potentially be varied, depending on virus and host factors (Coleman *et al.*, 1982; Jacoby *et al.*, 1979).

Infertility and unthrifty pups caused by RV must be differentiated from environmental and husbandry factors or infectious agents such as *Mycoplasma* or Sendai virus. Adult disease must be differentiated from toxicity, nutritional deficiency, and trauma. Diagnosis is made by the typical lesions, if present, virus isolation, and serology. Seroconversion to each virus (RV or H-1) can be detected by serum neutralization, hemagglutination inhibition, complement fixation, and immunofluorescence. Hemagglutination inhibition is currently the most commonly used means of antibody determination (Jacoby *et al.*, 1979).

Since RV infection is usually silent and persistent and can be transmitted either vertically or horizontally, effective control is best achieved by destroying the entire population, decontaminating, and repopulating with clean stock. Virus-free rats can be obtained from selected commercial vendors or by caesarean rederivation. Rederived progeny must be tested for vertically transmitted strains of virus. Colonies can be kept virus-free by limiting entry to seronegative, virus-free rats (as well as transplantable rat neoplasms or tissues), periodic serological testing, and adequate physical containment.

Although parvovirus infection of rats is usually inapparent, there can be adverse effects on the research usefulness of infected rats. Immunosuppression may exacerbate illness and mortality in latent carriers. The viruses often contaminate transplantable tumors and cell lines, can modify immune responsiveness or cause teratological effects. A decision to work with infected animals should be made carefully.

b. Other DNA Virus Infections. Rats are susceptible to rat cytomegalovirus, which has a predilection for the salivary and lacrimal glands. Infection is widespread among wild, but not laboratory rats (Jacoby *et al.*, 1979). Rats also seroconvert to

mouse adenovirus, but it is not known if infection is due to a mouse or rat strain of virus. Adenovirus-like inclusions have been reported in the intestine of rats treated with cancer chemotherapeutic agents (Ward and Young, 1976).

c. Sialodacryoadenitis Virus and Related Coronaviral Infections. Two strains of coronavirus have been identified as pathogens of laboratory rats: sialodacryoadenitis virus (SDAV) and rat coronavirus (RCV). Furthermore, rats are experimentally susceptible to the coronavirus of mice, mouse hepatitis virus (MHV). Coronaviruses are large, pleomorphic enveloped RNA viruses with surface peplomers or spikes that confer a corona-like appearance to the virion. Viruses of this group have complex antigenic interrelationships and cross-react extensively. Common antigens are shared by SDAV, RCV, and MHV, particularly by complement fixation, but antibody reactivity is highest with homologous virus. Sialodacryoadenitis virus and RCV represent different strains of the same virus, but whether different strains of the same virus or separate viruses, they are both important natural pathogens in rats. The significance of MHV for rats is not known, but the virus can replicate in the respiratory tract of intranasally inoculated rats (Taguchi *et al.*, 1979). Natural antibodies to MHV can occur in rats, but this is probably due to the closely related antigenicity of MHV to SDAV and RCV rather than natural MHV infection of rats (Barthold, 1984).

Clinical signs of SDAV infection vary widely in severity, but include blepharospasm, sneezing, porphyrin-pigmented nasal and ocular discharge, and cervical edema (Fig. 8). Some rats develop keratoconjunctivitis and other ocular lesions. Signs persist approximately one week, but ocular sequellae can be permanent. Acutely infected rats become anorectic, and estrus can cease temporarily. Infection is subclinical in weanling or older rats, but intranasally inoculated neonates die and sucklings develop lower respiratory disease.

Sialodacryoadenitis virus is highly contagious and spreads rapidly among susceptible rats by contact, aerosol, or fomite. Susceptible rats of any age can be infected. When enzootic within a colony, clinical disease occurs only in sucklings, since adults are immune. Infection is acute, lasting only about 1 week, at which time rats seroconvert with no carrier state. Maintenance of SDAV in a colony requires continuous introduction of susceptible stock as weanlings or newly introduced rats. The epizootiology of RCV is presumed to be similar to SDAV.

Within 2 days of intranasal inoculation, SDAV causes rhinitis followed by necrosis of the ductular and acinar epithelium of salivary and lacrimal glands, accompanied by intense inflammation and edema. Tracheitis and peribronchial lymphoid hyperplasia can also be found. Salivary glands appear swollen, pale, with interlobular and periglandular edema. Harderian glands are flecked with yellow-gray foci. One, some, or all of the salivary or lacrimal glands can be affected, with the exception of the sublingual glands, which are spared. Cervical lymph nodes become enlarged. Glandular repair ensues within 1 week, with squamous metaplasia of ductular epithelium and hyperplasia of acinar epithelium. The repair phase subsides within 30 days with minimal residual lesions. Interstitial pneumonia can occur in suckling, but not adult rats. Conjunctivitis, keratitis, corneal ulcers, synechia, hypopyon, and hyphema can arise due to lacrimal dysfunction. Eye lesions usually resolve, but can proceed to chronic keratitis, megaloglobus (Fig. 9), and retinal degeneration. Rat coronavirus infection causes rhinotracheitis and focal interstitial pneumonia. Salivary but not lacrimal gland infection is rare, but when present, resembles wild SDAV lesions. Infection with RCV also lasts approximately 1 week (Barthold, 1984; Jacoby *et al.*, 1979).

Nasal and ocular signs must be differentiated from those caused by mycoplasma, Sendai virus, pathogenic bacteria, excess ammonia, or hypovitaminosis A. Cervical swelling may

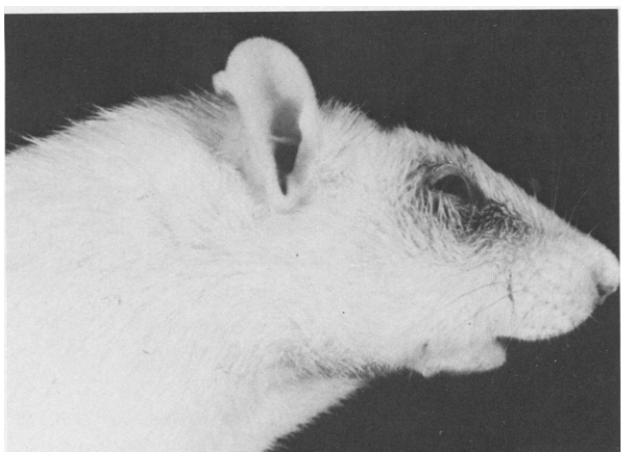

Fig. 8. Epiphora and swelling of the ventral neck in a rat naturally infected with SDAV. (From Barthold, 1984; courtesy of Hemisphere Publishing Corp.)

Fig. 9. Megaloglobus and hyphema in a young rat naturally infected with SDAV. (From Barthold, 1984; courtesy of Hemisphere Publishing Corp.)

also occur in immunosuppressed rats infected with *P. aeruginosa*. Microscopic SDAV lesions are characteristic. Mild lower respiratory tract lesions associated with RCV must be differentiated from those of Sendai virus or pneumonea virus of mice (PVM). Seroconversion or rising complement fixing antibody titers following acute disease is confirmatory. However, antibody may be low or undetectable with this method. Serum neutralization is another test that can be used, but the most sensitive antibody tests are immunofluorescence or ELISA. Either mouse or rat coronaviruses are used as antigen in these latter tests (Smith, 1983).

Rats can be kept free of SDAV and RCV if they are isolated and if newly introduced rats are immune or unexposed. Introduction of a single subclinically infected rat can precipitate epizootic disease among naive rats. If an outbreak occurs, the infection will run its course and die out within 3–4 weeks if new rats are not introduced into the room and if breeding is temporarily ceased. Routine disinfection of rooms and equipment is sufficient to destroy environmental sources of virus.

Sialodacryoadenitis virus lesions can be confused with or contribute to changes induced by test compounds or nutritional deficiencies, particularly vitamin A. Sialodoacyoadenitis virus disease can predispose to anesthetic death due to airway hypersecretion. Eye lesions resulting from SDAV infection can interfere with eye research. Both SDAV and RCV can potentiate other respiratory infections.

d. Sendai Viral Infection. Sendai virus commonly infects laboratory rats, but its clinical significance is less than in mice. Sendai virus is a parainfluenza 1 virus of the paramyxovirus family. Paramyxoviruses are pleomorphic, enveloped, labile RNA viruses. Sendai virus infection in rats is usually subclinical, but can be manifested as ruffled fur, dyspnea, or anorexia. A decrease in average litter size and runted pups is common during outbreaks in breeding colonies.

Sendai virus is highly contagious and disseminates rapidly. Outbreaks subside following development of an immune population, with the potential of recurrence several months later as the susceptible population enlarges. Sendai virus induces an acute respiratory infection with no natural carrier state. Excretion and transmission of virus occurs via the respiratory tract (Jacoby *et al.*, 1979).

Sendai viral lesions in the rat are similar to those in genetically resistant strains of mice. Following an initial necrotizing rhinitis, tracheobronchitis ensues within 1 week of infection. Lungs become consolidated and plum-colored in a patchy, primarily anteroventral, distribution. Necrotizing bronchiolitis, focal nonsuppurative interstitial pneumonia, and perivascular and peribronchial lymphoplasmacytic infiltrates are the hallmarks of the lower respiratory component. In the rat, acute lower respiratory lesions last only a few days and coincide with clinical disease. Repair of bronchial mucosa is effected through hyperplasia and squamous metaplasia. Residual lesions can last for months in the mouse, but probably not in rats. During the acute stage of Sendai virus infection, mucosal necrosis and inflammation can extend up the eustachian tubes to the middle ear. Bacterial otitis media is often preceded by Sendai viral infection in rodents (Brownstein *et al.*, 1981; D. G. Brownstein, unpublished observations, 1982).

Mild nonsuppurative pulmonary lesions can be seen with Sendai, PVM, RCV and to a lesser extent, SDAV. Sendai virus can be superimposed upon and contribute to respiratory mycoplasmosis. Squamous metaplasia of respiratory mucosa can mimic hypovitaminosis A and also occurs in mycoplasmosis. Virus isolation and seroconversion or rising titers to Sendai virus confirm a diagnosis based upon characteristic lesions and clinical signs. Sendai virus antibody can be accurately detected by complement fixation and hemagglutination inhibition, but newly developed immunofluorescence and ELISA are replacing these methods (Smith, 1984).

Rats can be kept free of Sendai virus in a similar manner as with SDAV and RCV. Recovered Sendai virus antibody-positive stock can be utilized as breeders to reestablish a virus-free population. Pups born of these parents will be virus-free and antibody negative, following decay of their maternal antibody. Mice and hamsters can serve as sources of infection with paramyxoviruses.

Sendai virus infection, although usually subclinical, can cause significant pulmonary changes and act as a copathogen with mycoplasma or bacteria. Squamous metaplasia and hyperplasia induced by these agents influence respiratory carcinogenesis and interpretation. Sendai virus can cause immunosuppression for prolonged periods in rats (Garlinghouse and Van Hoosier, 1978).

e. Pneumonia Virus of Mice Infection. Pneumonia virus of mice is a pneumovirus of the paramyxovirus family and thus shares many features in common with Sendai virus. It has not been associated with clinical disease in the rat or mouse under natural conditions. Several rodent species, including hamsters, guinea pigs, and gerbils, seroconvert to PVM under natural conditions. The virus seems to spread rapidly among susceptible rodents, based on seroconversion. It is presumed to be transmitted in a manner analogous to Sendai virus.

The pathogenesis of PVM has been investigated in mice, but has not been thoroughly examined in rats, even though it produces more significant pulmonary lesions in rats than mice under natural conditions. In the mouse, PVM replicates in the upper respiratory mucosa without obvious cytolysis following low-dose exposure (D. G. Brownstein, unpublished observations, 1982). Viral antigen and cytolytic lesions are seen in the bronchial and bronchiolar epithelium as well as alveolar walls following apparently higher doses of virus in a pattern reminiscent of Sendai virus. The ensuring lesion, like that of Sendai

virus, is necrotizing bronchiolitis and interstitial pneumonia (Carthew and Sparrow, 1980). The active infection lasts about 1 week, after which time lymphocytic infiltration of peribronchial and perivascular tissues and focal interstitial pneumonia can persist for several weeks. Rat lungs develop prominent nonsuppurative perivascular and mild interstitial inflammation in response to both experimental and natural PVM infections (D. G. Brownstein, unpublished observations, 1982; Vogtsberger *et al.*, 1982).

Infection is usually diagnosed retrospectively in rats, where pulmonary lesions are observed following seroconversion to PVM in the absence of other respiratory pathogens. Pulmonary lesions mimic those of Sendai virus or possibly coronaviruses. Antibody to PVM has usually been detected by hemagglutination inhibition or serum neutralization, but these tests are being replaced by immunofluorescence and ELISA. Hemagglutination inhibition is least expensive and highly accurate, however (Smith, 1983). Pulmonary lesions induced by PVM can interfere with the interpretation of experimental studies, or possibly pulmonary function, although this has not been established.

f. Hemorrhagic Fever with Renal Syndrome (HFRS). Hemorrhagic fever with renal syndrome, Korean hemorrhagic fever, or muroid virus nephropathy is a human disease complex caused by one or more serologically related bunyaviruses, including Hantaan virus. This syndrome is mentioned here because of its emerging eminence as a major zoonotic disease in which several species of the suprafamily Muroidae, including laboratory rats, can serve as carriers. The virus is transmitted to man by excretions and aerosols from the lungs, saliva, and urine of healthy rodent carriers. Pathology, if any, in rats has not been described. The disease in humans includes proteinuria, azotemia, and, less often, petechiae, hemoconcentration, hypotension, and renal failure. Human mortality can occur, but recovery with immunity to reinfection is usual. Hemorrhagic fever with renal syndrome viruses cause human disease throughout much of Eurasia, but on the basis of serological evidence, HFRS viruses exist world-wide in feral rodents and humans, including the United States. Transmission of HFRS to laboratory personnel by silently infected albino laboratory rats (*Rattus norvegicus*) has been documented in Japan and Belgium. Virus-infected lung and kidney cell lines provide excellent sources of antigen for sensitive immunofluorescence tests, but serological monitoring of laboratory rats for HFRS virus antibody is not currently a common practice (Gajdusek *et al.*, 1982; Anonymous, 1982).

g. Other Virus Infections. Rats harbor endogenous retroviruses or genomic retrovirus sequences that (in contrast to the mouse) are of minimal significance in terms of natural disease. A number of endogenous rat sequences have been artificially recombined with mouse leukemia virus sequences or naturally recombined with other rat retrovirus sequences to form defective rat sarcoma viruses, for example Kirsten and Harvey rat sarcoma viruses, which are experimentally oncogenic to rats, mice, and hamsters (Shih and Scolnick, 1980). Natural seroconversion to reovirus-3 and mouse encephalomyelitis virus have been noted with no apparent disease. An enterovirus with neurotropic properties has been isolated from naturally infected, asymptomatic rats. Although rats can be experimentally infected with ectromelia and lymphocytic choriomeningitis virus, these agents are not of practical significance as natural infections of rats. Testing for antibody to these agents is therefore a vacuous exercise. A number of other viruses or viruslike agents have been isolated or incriminated as causes of disease in rats, including rat submaxillary gland virus, Novy virus, enzootic bronchiectasis agent, gray lung virus, and wild rat pneumonia agent. These agents, if extant at all, are of dubious importance in contemporary laboratory rat populations (Jacoby *et al.*, 1979). Recently, clinically silent infection with an unidentified poxvirus has been found in Soviet rats. Microscopic necrotizing and inflammatory changes were restricted to the nose (Kraft *et al.*, 1982). The agent is believed to be cow pox virus.

5. Parasitic Diseases

Laboratory rats are host to far fewer parasites than wild rats, since husbandry practices interrupt complex life cycles and caesarean rederivation has simply eliminated many agents altogether. Control of these agents in infected colonies is also best achieved by these means. This section will mention only agents that have been reported in laboratory rats, although most are unlikely or rare. Table IX outlines selected treatment regimens for control of common metazoan parasites, but the reader must beware that these drugs may render the treated rat useless for experimental work. Decisions to treat rather than eliminate infected rodents must be made with care, in concert with the needs of the scientific investigator.

a. Protozoal Infections. Several flagellates can parasitize, rats but few are truly pathogenic. Hemoflagellates (*Trypanosoma cruzi, T. lewisi*) occur in wild rats, but these agents require anthropod vectors. Nevertheless, *T. lewisi* can be encountered if a colony is infested with rat fleas. *Trypansoma lewisi* is only mildly pathogenic even in heavy infections, and infection is short term. Rats harboring this organism become ill when stressed or immunosuppressed. Enteric flagellates (*Tritrichomonas* sp., *Tetratrichomonas* sp., *Pentatrichomas* sp., *Trichomitis* sp., *Hexamastix* sp., *Enteromonas* sp., *Retortamonas* sp., *Chilomastix* sp., *Monocercomonoides* sp., and *Octomitus* sp.) are very common but nonpathogenic in laboratory rats. *Giardia muris* and *Spironucleus* (Hexamita) *muris*

Table IX

Selected Treatment Regimens for Common Rat Parasites[a,b]

Agent	Compound	Application
Oxyurids	Piperazine citrate	3–10 gm/liter drinking water for 7 days, stop 7 days, repeat 7 days
	Piperazine adipate	0.5 gm/liter drinking water for 3 days
	Pyruvinium pamoate	3 mg/liter drinking water or 1.2 mg/kg feed for 4 weeks
	Dichlorvos	0.5 mg/gm feed for 1 day
Trichosomoides	Methyridine	100 mg/g sc, single dose
Cestodes	Niclosamide	100 mg/kg po, single dose
Ectoparasites	Rotenone	0.5–1.0% dust
	Silica dust	With or without pyrethrin
	Dichlorvos strip	24 hr/week on cage top for several weeks
	Dichlorvos pellets	2–6 gm of pellets in bedding for 5 days, change bedding and cage, repeat at 12 days, change and repeat at 1 month
	Diazinon	0.25% dip
	Malathion	3–5% dust, up to 0.5% spray or up to 2% dip, repeat at 12 days
	Ronnel	up to 0.5% dip
	Carbaryl	1% spray

[a]Information kindly supplied by Dr. J. W. Streett, Yale University.

[b]A number of these compounds have adverse effects on the experimental usefulness of treated rats and therefore should be used with discretion and full knowledge of the investigator.

are also common and considered potentially pathogenic, causing unthriftiness, diarrhea, and intestinal lesions in severely affected animals. However, these are generalizations made from other species, since natural clinical disease has not been reported among rats.

Sporozoan parasites, common in wild rats, are encountered only on occasion in laboratory rats. *Hepatozoon muris* infection is usually subclinical, but severely affected rats can have leucocytosis, splenomegaly, anemia, hepatic degeneration, emaciation, or death. Infection requires ingestion of the vector, *Laelaps echidninus*. Toxoplasmosis (*Toxoplasma gondii*) is subclinical in rats, requiring the ingestion of cat feces or contaminated tissues containing asexual stages, but can also be transmitted transplacentally. Intracellular *T. gondii* organisms occur in several tissues, particularly lung and reticuloendothelial organs, and cysts are found in the central nervous system. *Sarcocystis muris*, once common but now rare in laboratory rats, has a life cycle similar to *T. gondii*. Cysts are found in the muscle. *Frenkelia* sp. has been described in a colony of laboratory rats, causing large thin-walled cysts and inflammation in the central nervous system, but no clinical signs. *Encephalitozoon cuniculi* is apparently now uncommon in laboratory rats and seldom induces signs or lesions. Its prevalence and significance are far greater in the laboratory rabbit. Single or clusters of small spores form within cysts in a number of tissues. Nonsuppurative inflammation can be found in the brain and kidneys. Unlike *T. gondii*, *E. cuniculi* organisms are gram-positive. *Encephalitozoon cuniculi* can be transmitted via contaminated transplantable tumors, tissue, or tissue fluids from infected rats. Intestinal sporozoans include *Eimeria nieschulzi* and *E. miyairii*, which infect the small intestine, and *E. separata*, which infect the large intestine of wild rats. They are exceedingly rare or nonexistent in laboratory rats.

Pneumocystis carinii, once considered to be a yeast but now considered a sporozoan, is an ubiquitous organism that infects the lung of a variety of species, including laboratory rats. Prevalence of infection is high within infected colonies, but prevalence among various geographic areas in unknown. It seems to be very common among both conventional as well as barrier-reared rats. Normally *P. carinii* is not pathogenic, but infected rats can develop disease after several weeks of repeated steroid injections and starvation, particularly in younger rats. Remarkably, rats from many commercial sources can be so treated and shown to be infected, even if free of other pathogens. Under these circumstances, the lungs are expanded and rubbery. Alveolar walls are thickened and hypercellular, and alveoli are distended with proteinaceous, eosinophilic foamy material containing macrophages and organisms in different stages of development. Tissues can be stained with special stains, such as methanamine silver or periodic acid–Schiff, to visualize the organisms, which are not visible with hematoxylin and eosin.

Other protozoa, including *Entamoeba muris* and *Balantidium coli*, are found in the large bowel of rats, but are not pathogenic. The reader is referred to more exhaustive coverage of protozoa for detailed means of diagnosis (Flynn, 1973; Hsu, 1979).

b. Nematodiasis. *Syphacia muris* is a common oxyurid (pinworm) of the cecum and colon. Embryonated eggs are passed in the feces or deposited on the anus. Rats are infected by ingestion of eggs or by larval migration into the colon from the anus. *Syphacia muris* closely resembles *S. obvelata*, the mouse pinworm. Both species of *Syphacia* can patently infect rats or mice, but preference seems to be shown for the homologous host. Another common oxyurid of rats and mice is *Aspicularis tetraptera*, which is also found in the cecum and colon. *Aspicularis* eggs are nonembryonated, and are not deposited near the anus. Depending on parasite species, the prepatent period is 18–23 days. Diagnosis is achieved by examination of large bowel contents (particularly the cecum and proximal colon) for nematodes, feces for ova, and, in the case

of *Syphacia*, cellophane tape impressions of the anus for ova. Speciation is easily achieved by the differential morphology of ova. *Aspicularis* ova are symmetrically ellipsoidal, while *Syphacia* ova are flattened on one side. Since eggs are extremely resistant to environmental factors and disinfectants, and autoinfection can occur with *S. muris*, infection is difficult to eradicate. Anthelminthic treatment ameliorates infection (Table IX), but all rats must be treated simultaneously and the room must be thoroughly cleaned. Despite these actions, reinfection frequently occurs. Caesarean rederivation and subsequent strict sanitation are the most effective means of control. Pinworms are generally considered nonpathogenic, but heavy parasite loads can be deleterious to the host. Concern has been raised regarding the effects of these agents on research but no effects have been documented in the rat.

Trichosomoides crassicauda is common in some rat colonies but is not generally encountered in commercially raised animals. It inhabits the transitional epithelium and lumen of the urinary tract. The small male resides within the reproductive tract of the female, whose anterior end embeds within the mucosa. Embryonated ova with bipolar opercula are shed in the urine. Following ingestion, eggs hatch in the stomach, larvae penetrate the gastic wall, then migrate via the lungs, kidney, and ureter to the urinary bladder. Migrating larvae can incite eosinophilia and formation of granulomata, particularly in the lung. Drug treatment (Table IX) is effective, as is cesarean rederivation. Parasites do not incite an inflammatory response in the bladder, but can serve as a nidus for calculi or enhance natural and experimental bladder carcinogenesis.

Other nematodes are common in wild but rare in laboratory rats. *Trichinella spiralis* adults occur in the intestine, and larvae migrate extensively, encysting in muscle. Transmission is effected by ingestion of contaminated meat. *Capillaria hepatica* eggs are ingested, hatch, and migrate through the cecum to the liver through the portal veins. Adults mature and lay characteristic bipolar operculate ova in the liver, where they lie dormant until ingested by a carnivore. *Gongylonema neoplastium* burrows in the squamous epithelium of the tongue and anterior stomach, where it incites a proliferative response or ulceration. The life cycle involves an intermediate insect host. *Angiostrongylus cantonesis*, which requires the ingestion of a mollusk intermediate host, infects the brain and lung. The intestinal nematodes *Heterakis spumosa*, *Nippostrongylus brasiliensis*, *Strongyloides ratti*, and *Trichuris muris* lack intermediate hosts, but rarely occur naturally in laboratory rats. None is known to be very pathogenic. Further details on nematode diagnosis and ova morphology are available (Flynn, 1973; Hsu, 1979; Oldstone, 1967).

c. Cestodiasis. Rats can serve as the intermediate host for *Taenia taeniaformis*, the cat tapeworm. Following ingestion of ova, oncospheres migrate to the liver and form strobilocerci (*Cysticercus fasiolaris*). Host connective tissue capsules can give rise to sarcomas. This parasite has been used as a model of parasite-induced oncogenesis in the rat. *Cysticercus fasciolaris* is on occasion encountered in laboratory rodents with contaminated food or bedding.

Hymenolepis nana and *diminuta* infect the small intestine of several species, including the rat. *Hymenolepis nana*, the dwarf tapeworm, has a 1-mm wide segmented body ranging from 7 to 100 mm in length. The life cycle may be either direct or indirect. Following ingestion of ova, oncospheres penetrate the intestinal mucosa, form cysticercoid larva, then emerge into the lumen and mature into adults that shed eggs in the feces. Nonimmune hosts can be autoinfected; eggs are produced, hatched, and complete their life cycle within the intestine of a single host. The indirect cycle involves intermediate hosts, such as grain beetles or fleas, in which cysticercoid larvae form and await ingestion by the definitive host. *Hymenolepis diminuta* is 3–4 mm wide and 20–60 mm long. The eggs are passed in the feces but must be ingested by an intermediate host (flour beetle, flea, moth), which in turn must be ingested by the definitive host to complete the life cycle. Enteritis can occur in heavy infestations, which are most likely to be encountered with *H. nana*. Both can infect primates, including humans. *Hymenolepis nana*, which does not need an intermediate host and can cause autoinfection, is a particular public health hazard. Anthelmintic treatment is effective (Table IX). Diagnosis of *Hymenolepis* is made by finding ova containing hexacanth embryos in the feces or adults in the lumen of the bowel. Differential features are detailed elsewhere (Hsu, 1979).

d. Insect Infestation. Two anopluran (sucking) lice infest wild rats, *Polyplax spinulosa* (the spined rat louse) and *Hoplopleura pacifica* (the tropical rat louse). The latter has not been reported among laboratory rats. *Polyplax spinulosa* is now rare in most laboratory rat populations. It completes its entire life cycle within the fur of the host and is transmitted by direct contact. Infested rats are unthrifty, irritable, pruritic, restless, and anemic. *Polyplax spinulosa* can transmit a number of infectious agents, which include *Hemobartonella muris* and *T. lewisi*. Diagnosis is made by identifying organisms in the fur.

Rat fleas (*Xenopsylla* sp., *Leptopsylla* sp., and *Nosopsyllus* sp.) are rare in laboratory rats, since their life cycles are usually interrupted by proper sanitation. Fleas can transmit other agents and can serve as the intermediate host of *H. nana* and *diminuta* (Flynn, 1973; Hsu, 1979; Oldstone, 1967). See Section III,A,5,e for methods of control.

e. Arachnid Infestation. Mesostigmate mites can be encountered on occasion in laboratory rats. They are rapid bloodsuckers and have a nonselective host range. They are on the

host only during feeding, spending much of their life cycle secreted in the environment. Diagnosis must be attained by finding engorged mites on bedding, cages, and crevices. Other hosts, including humans, fall prey to their painful and irritating bites. The most important mesostigmate is *Ornithonyssus bacoti* (tropical rat mite). Heavily infested colonies contain debilitated, anemic rats with reduced reproductive efficiency and occasional deaths. *Liponyssoides sanguineus* has not been reported in laboratory rats, but may be unrecognized because of its similarity to *Ornithonyssus* sp. *Laelaps echidninus* (spiny rat mite) does not adapt well to the laboratory rat environment unless husbandry is poor. It is a vector of *Hepatozoon.*

Prostigmate mites have a more selective host range and spend their life cycle in the fur (or follicles) of the host. *Radfordia ensifera,* the rat fur mite, can be common in some rat colonies. This mite produces few ill effects, but heavy infestations can induce self-inflicted trauma (Flynn, 1973; Hsu, 1979; Oldstone, 1967). *Demodex* sp. is also encountered, but its prevalence is unknown, since it lives deep within hair follicles and sebaceous glands where it produces minimal lesions (Walberg *et al.,* 1981).

Astigmate mites include the mange mites, which have a selective host range and complete their life cycle on the host. *Notoedres muris,* the ear mange mite, infests the skin of the ear and to a lesser extent, the nonglabrous regions of the body. This mite burrows in the cornified epithelium, and elicit a pruritic dermatitis (Flynn, 1973; Hsu, 1979). It is seldom seen in contemporary rat colonies.

Control of ectoparasites is gained by preventing the introduction of infected animals (including wild rodents), sanitation, treatment of rats, and, in the case of mesostigmates and fleas, treatment of the environment (Table IX). Treatment seldom completely eliminates infestation and can have an adverse effect on the usefulness of the treated rats for research. Repopulation or caesarean rederivation and subsequent prevention by proper management is the best approach.

6. Fungal Diseases

Deep mycoses are generally not considered to be significant natural diseases in laboratory rats. Pulmonary aspergillosis is occasionally observed in rats and mice, particularly when immunocompromised. *Aspergillus* sp. can be readily isolated or observed in affected lung tissue with selected histochemical techniques. Pulmonary lesions consist of miliary granulomata containing Langhans giant cells in areas with characteristic uniformly branched, septate hyphae. Phycomycotic encephalitis has been reported as a natural disease in 2- to 4-week-old rats. Phycomycotic fungi have thick, irregularly branched, nonseptate hyphae in tissue.

Dermatomycosis is probably more likely to be encountered than deep mycosis, but is also rare in laboratory rats. Ringworm in rats is restricted largely to infection by *Trichophyton mentagrophytes.* Infected rats can be lesionless carriers or display patchy alopecia and erythema with scale, papule, or pustule formation. Fungal elements are visible within the stratum corneum and hair follicles. Ecothrix spore formation in hair shafts can be present. The disease can be diagnosed by examination of skin scrapings treated with 10% potassium hydroxide, histological sections of skin prepared with fungal stains, or isolation (Weisbroth, 1979).

B. Noninfectious Diseases

1. Metabolic/Nutritional Diseases

a. Genetic Anomalies. Genetic anomalies in coat color and character, organ development, immune responsiveness, obesity, hypertension, metabolism, and others have been identified and in many cases developed as specific characteristics of various stocks and strains. The reader is referred to several more reviews for further information (Altman and Katz, 1979; Hansen *et al.,* 1981; Robinson, 1965, 1979). Some strains naturally develop disease syndromes, such as Brattleboro rats with diabetes insipidus, while other strains have less obvious characteristics but react uniquely to different research variables. The researcher must be aware of these variations and judiciously select the appropriate stock or strain to accomplish the goals of the experiment.

Each stock, strain, substrain, and even group of rats that has drifted from its parental stock can develop its own unique diseases, manifest its own incidence and rate of development of various spontaneous lesions, and react in different ways to infectious and research variables. Expression of genetic traits is further influenced by extraneous factors such as husbandry and diet.

b. Nutritional Deficiencies. More is known about the nutritional requirements and deficiencies of rats than perhaps any other species. Natural deficiencies of single dietary components are rare and seldom recognized. This is because rats effectively store fat-soluble vitamins (and B_{12}), manufacture vitamin C, and fulfill much of their requirement for B vitamins by coprophagy. Furthermore, some deficiencies are influenced or compensated for by other dietary elements. The most common signs of nutritional deficiency are vague, such as poor hair coat, reduced weight gain or growth, diminished fertility, and susceptibility to infectious disease. Commercially available rodent diets effectively supply balanced diets to rodents, but diets should not be utilized beyond their expiration date and ideally should be kept in cold storage. Several components deteriorate with prolonged storage, heating, or sterilization, including lysine, vitamins A and E, and, to a lesser extent,

riboflavin and thiamin. Nutritional requirements vary with the genetic strain, stage of life, and microbiological association. Axenic or specific pathogen-free (SPF) animals can lack gut microflora necessary for supplying certain vitamins, particularly vitamin K. They must receive supplemented diets both because they need more and because autoclaving or pasteurization reduce the levels of certain essential nutrients. Antibiotic treatment, which affects intestinal microflora, can reduce the bacterial source of vitamins by coprophagy. Nutritional adequacy goes far beyond the needs of the rat, since the composition of nutritionally adequate diets is known to profoundly influence the biological responsiveness to research manipulation, infectious disease, and expression of spontaneous lesions (National Research Council, 1978; Rogers, 1979).

The signs of nutritional deficiency are often vague and must be differentiated from other syndromes. Hemorrhage due to hypovitaminosis K can be confused with RV disease (which in itself can be precipitated by nutritional deficiency). Infertility or poor production can be caused by RV, SDAV, Sendai virus, *M. pulmonis,* or nuturitional deficiency. Squamous metaplasia in the salivary and lacrimal glands of rats recovering from SDAV or in the respiratory tract of rats infected with Sendai virus or *M. pulmonis* must be differentiated from hypovitaminosis A, which in turn predisposes to respiratory infections. Environmental factors, such as excess heat or low humidity, contribute to infertility or skin disease which mimic hypovitaminosis E or other deficiencies. Ordinarily mild infectious diseases can become severe, such as pseudotuberculosis, due to nutritional deficiency. The diagnosis of many rat diseases therefore requires review of nutritional practices.

2. Management-Related Diseases (Nonnutritional)

Practices other than nutrition can also influence or cause disease in rats. Sanitation is of obvious import. Cage design, population density, and temperature influences have been mentioned in Sections II,D and E. Outbreaks of opportunistic infectious disease, such as pseudotuberculosis, can be precipitated by sudden fluctuations in temperature and humidity. *Pseudomonas aeruginosa* can be introduced and spread within a colony through inappropriate sanitation, particularly of water bottles. Refilling water bottles at a faucet in the animal room is not advised for this reason. Bottles and sipper tubes should be sanitized and water should be acidified or chlorinated. Excessive ammonia from dirty bedding, overcrowded cages, or poor ventilation predisposes to respiratory infections, particularly mycoplasmosis.

A number of management-related syndromes can occur that are not related to sanitation or infectious disease. Rats adapt well to automatic watering systems, but animals unfamiliar with these devices can become dehydrated. Similarly, malfunction of these systems and blockage of water bottle sippe tubes with metal filings or other detritus can have a similar effect. Rats maintained for long periods on wire-bottom cages develop foot sores, which can result in severe lesions, discomfort, hemorrhage, and anemia. Aging rats must be examined periodically for overgrowth of their incisors, a very common problem in rats held long term. Teeth must be carefully clipped, avoiding splitting or shattering. Dusty bedding and food can result in foreign body pneumonia. Plant, mineral, and even bone fragments can be found in sections of lung obtained from rats under these circumstances. Softwood shavings used as bedding can cause intestinal impaction, particularly in young rats. High ambient light, or even light levels within the recommended range, can cause retinal degeneration and cataracts in albino rats. Rats maintained near ceiling light fixtures develop retinal degeneration, while those near the floor are spared. Low relative humidity (<40%) in concert with high temperature and other factors can cause one or more annular constrictions of the tail skin (ringtail) or toes. The segments distal to the constrictions swell or undergo dry gangrene (Fig. 10). This is most apt to occur in suckling or preweanling rats and in rats kept in wire-bottom cages. Increasing humidity, reducing air turnover rate, placing rats in solid-bottom cages, and providing nesting material for nursing dams and their litters are all ameliorative for this condition.

Axenic rats normally have enlarged ceca, which become approximately five times normal size. There is net efflux of fluid into the lumen in response to increased osmotic pressure of luminal content, reduced availability of ions needed for mucosal solute-coupled water resorption and vasoactive compounds which exert their effect on smooth muscle tone. Cecal enlargement can interfere with reproduction. Dietary manipulation can reduce the enlargement substantially (Foster, 1980). The effects on smooth muscle tone become pronounced in aging axenic rats, which can develop progressive cecal enlargement due to atony. Volvulus and torsion can result.

The reader must be cognizant of the much larger list of en-

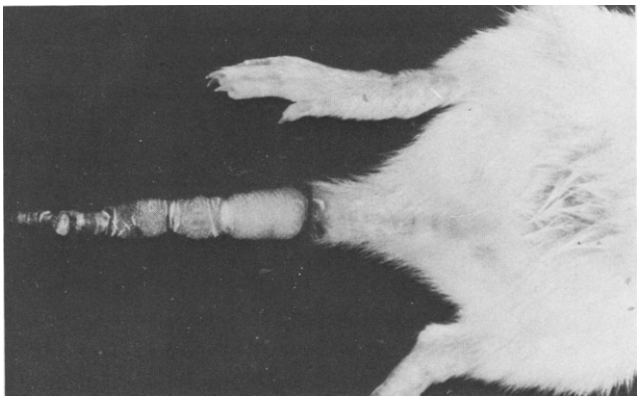

Fig. 10. "Ringtail" in a preweanling rat. Annular constrictions and dry gangrene of the tail.

vironmental variables that significantly alter physiological responsiveness of test animals (Baker *et al.*, 1979a; Gralla, 1981).

3. Traumatic and Iatrogenic Diseases

Unlike the mouse, traumatic skin disease in the rat is more likely to be due to self-inflicted trauma than fight wounds. Rats infested with ectoparasites or dermatophytes can develop excoriative dermatitis due to self-inflicted trauma. Roughly symmetrical ulcerative dermatitis over the dorsal and lateral neck and shoulders has been reported (Fig. 11). The etiology remains to be determined, but coagulase-positive *Staphylococcus aureus* is consistently isolated from the skin lesions, and colonies of gram-positive cocci are microscopically visible on the surface of the lesion. Attempts to induce the disease in other rats with this agent have yielded inconsistent results (Fox *et al.*, 1977).

The rat is often considered "resistant" to infection and accordingly submitted to a variety of experimental procedures without regard to aseptic techniques. Peritonitis and wound infections are often found under such conditions. Inappropriate handling of large rats by the tips of their tails will cause the skin to tear and slip off when they struggle.

Adynamic ileus occurs in rats given intraperitoneal injections of anesthetic preparations containing chloral hydrate or related compounds (Fig. 12). For variable periods up to 5 weeks after treatment, rats become lethargic, anorectic, and constipated with distended abdomens that may lead to death. Gross necropsy findings reveal segmental atony and distension of the bowel, usually jejunum, ileum, and cecum. Focal serosal inflammatory changes and fibrosis are seen microscopically (Fleischman *et al.*, 1977). This syndrome mimics megaloileitis (Tyzzer's disease), but can be differentiated on the basis of history, lack of characteristic bacteria in tissues, and lack of liver and heart lesions. In addition, it should not be confused with the bowel dilatation observed in axenic rats.

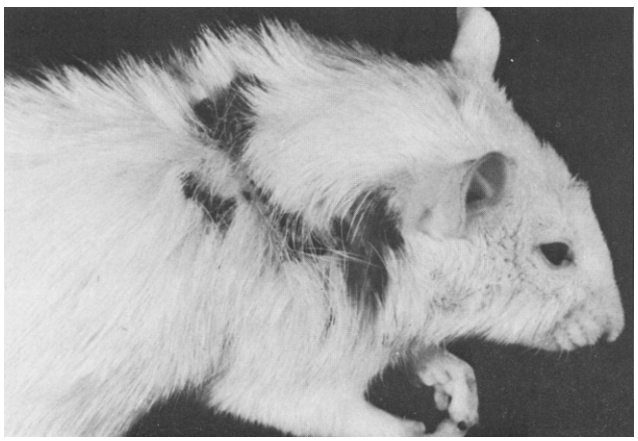

Fig. 11. Ulcerative dermatitis in a rat, depicting the typical location for lesions in this syndrome.

Fig. 12. Adynamic ileus in a rat which received a previous intraperitoneal inoculation of chloral hydrate.

C. Neoplastic Diseases

Space exists in this chapter to only summarize the more frequently reported tumors in the rat, and the topic will not include experimentally induced tumors. The reader is encouraged to refer to the articles and monographs referenced in the text for more complete information.

There have been numerous studies done to assess the incidence of neoplasia in various stocks and strains of rats (Burek, 1978; Coleman *et al.*, 1977; International Agency for Research on Cancer, 1973, 1976; MacKenzie and Garner, 1973; Ringler and Dabich, 1979; Sher, 1982). Some of these have led to the development of animal models and provided baseline data for experimental carcinogenesis work for which the rat is regularly used. There is a wide range of values reported in regard to the prevalence and type of tumors found in a particular rat stock or strain. These differences are a function of genetic and environmental influences and differences in sample selection. Nutrition is well known as a factor that influences the development of neoplastic disease. For instance, it has been shown that a high fat diet enhances tumorigenesis, that the protein–calorie ratio influences the occurrence of some tumors, and that restricted food intake of postweanlings for 7 or more weeks decreases tumor risk (Ross and Bras, 1971).

Most of the surveys on tumor occurrence in rats either state

nothing in regard to the presence of enzootic infectious disease or acknowledge that infectious diseases were present in the population. In many instances, infectious diseases can significantly influence data on tumor risk because of their effect on longevity, preneoplastic changes, and masking of small tumors.

With the exception of mammary fibroadenomas in many stocks and testicular tumors in F344 rats, the prevalence of tumors is quite low in rats under 18 months of age. Accordingly, the age at which rats are surveyed is very important in defining the incidence of neoplasia in a particular stock or strain.

The F344 rat is used in the bioassay program of the National Cancer Institute and is widely used in toxicology studies. It has been selected for these types of studies because of its small size, longevity, and low incidence of most tumors. However, this strain has a moderate to high incidence for some tumors, such as those of the testis, mammary gland, uterus, and hematopoietic and endocrine systems (Sher, 1982). A Sprague-Dawley-derived stock that is commonly used in carcinogenesis studies is the CRL:CD(SD)BR rat. This stock has a high incidence of mammary tumors and pituitary adenomas.

1. Skin and Mammary Gland

Papillomas and squamous cell carcinomas occur infrequently, but have been reported in many stocks of rats. Squamous cell carcinomas are usually located on the face and head, and tend to be highly invasive but not metastatic. Most of these arise as sebaceosquamous tumors from the glands of Zymbal in the ear. Similar tumors occur on the prepuce and clitoris. In females from most stocks and strains, the mammary gland is the most frequent site of neoplasia, with incidences of 30–60% being common. These tumors occur in males but much less frequently. Benign fibroadenomas are the most common type, but adenocarcinomas may also occur. Fibroadenomas have ductal epithelium and periductular connective tissue components and tend to be less vascular than carcinomas. Adenocarcinomas can metastasize to regional lymph nodes and the lung. Tumors of the mammary gland, which may arise at any site from the neck to the inguinal region, often attain a very large size (Fig. 13).

2. Digestive System

The prevalence of tumors in the alimentary tract is extremely low, with most cases involving the colon. Although hepatic tumors are readily induced by chemical carcinogens, very few spontaneously occurring malignant neoplasms have been reported. More commonly occurring are neoplastic nodular lesions. The term "neoplastic nodule" is used to describe circumscribed nodules of proliferating hepatocytes that compress the parenchyma (Altman and Goodman, 1979). There is some

Fig. 13. Mammary fibroadenoma in an aged rat.

controversy as to whether these nodules are in all cases neoplastic. However, there is evidence that some develop into carcinomas.

3. Hematopoietic System

Leukemia is rare in many stocks and strains of rats. However, mononuclear cell leukemia is very frequently seen in F344 and WF rats. In one survey of male F344 rats, an incidence of 16% proved leukemia second in occurrence only to the testicular interstitial cell tumors. The leukocyte count in 9 leukemic rats ranged from 68,400 to 323,000 cells/μl with an average of 143,190 (Coleman *et al.*, 1977). Other studies indicate a similar incidence for the WF rat. In the BN/Bi rat, myelomonocytic leukemia has been reported to be the most common lymphoreticular tumor, with female and male incidences of 5 and 11%, respectively (Burek, 1978). Both types of leukemia occur in rats over 18 months of age.

4. Endocrine System

Neoplasms of the pituitary occur frequently with a higher proportion in females. Surveys have shown an incidence of 15–30% in F344 rats, 18% in OM (Osborne-Mendel) rats, and 3–13% in Sprague-Dawley rats. The most common pituitary tumor is the chromophobe adenoma. These are benign tumors that frequently become quite large, and, due to compression of the brain by the tumor, hydrocephalus and neurological signs may occur. They appear dark due to hemorrhagic areas arising from a vascular sinusoid component of the tumor. Clinical manifestations may include neurological signs or cachexia, but these are often absent.

Pancreatic islet cell tumors are common in some stocks and strains. Surveys have reported a 6% incidence in F344 rats, 4.4% in Holtzman rats, and a 1–2.9% in Sprague-Dawley stocks.

Tumors of the thyroid are usually parafollicular cell adenomas. These benign tumors occur in many stocks with an incidence varying between 1 and 7%. Follicular cell carcinomas, which occur infrequently, can metastasize to the lung.

Adenomas of the adrenal cortex are very common in several strains. In at least three strains, BUF/N, M520/N, and OM/N, over 40% of the females and 20% of the males have cortical tumors. Pheochromocytomas are the most common medullary tumors. They are particularly common in the BUF/N, F344/N, M520/M, and WN/N strains, and, unlike cortical tumors, they appear more often in males.

5. Central Nervous System

Tumors of the brain have been found to occur rarely in those studies that included examination of the CNS. Less is known about the prevalence of tumors in the CNS than in other systems because many studies have not included brain and spinal cord examination. The astrocytoma is the most commonly reported tumor of the CNS. Less often reported tumors include oligodendroglioma, meningioma, ependymoma, and granular cell tumors.

6. Respiratory System

Primary tumors of the lung are rare in the rat. The most frequently reported types are bronchogenic carcinomas, carcinomas, sarcomas, and adenomas.

7. Urinary System

Tumors of the bladder are rare in most stocks and strains of rats. However, the BN/Bi strain has an unusually high incidence of carcinomas of the bladder and ureter. Males of this strain have been reported to have a 35% incidence of bladder carcinoma, while 22% of females have carcinoma of the ureter (Burek, 1978). Nephroblastomas, renal tubular adenomas, and adenocarcinomas have been reported in the rat kidney.

8. Reproductive System

Tumors of the prostate are common in the AXC rat. In one report, adenocarcinoma of the ventral prostate was histologically detected in 70% of 30- to 46-month-old AXC rats. Prostatic tumors are reportedly uncommon in most stocks and strains of rats. Reportedly, however, some of these surveys did not include adequate sectioning to locate small adenomas and carcinomas.

Interstitial cell tumors are the most common testicular tumor type. Nearly all aged F344 rats and the majority of ACI/N rats develop these tumors, but the tumor is rare in most other strains.

Tumors of the vagina and cervix are rare, except in aged BN/Bi rats. Tumors of the uterus and ovary are common in several strains of rats. One OM strain was reported to have a 33% incidence of granulosa cell tumors, and a high incidence of endometrial tumors has been reported in the F344 and M520 strains.

D. Miscellaneous Diseases and Lesions

1. Congenital/Hereditary Lesions

Congenital disorders in the rat are infrequently reported. This is probably a reflection of two factors; the first being that evidence indicates the incidence is extremely low in outbred stocks, and the second being that commercial/institutional suppliers select against breeders who have produced abnormal young. The genitourinary system and the eye have been most associated with congenital disorders.

One substrain of the AXC rat has a greater than 25% incidence of unilateral renal agenesis and hydronephrosis (Fujikura, 1970). A high incidence of unilateral and bilateral hydronephrosis has been found in BN/Bi rats (Cohen *et al.*, 1970). Pseudohermaphroditism has been reported in rats that are phenotypically female but who have an XY karyotype. Testicles are present either in the abdomen or inguinal canal. This condition is due to a sex-linked recessive gene that is expressed by an insensitivity of tissues to androgens (Bardin *et al.*, 1970).

A number of ocular disorders have been reported in the rat. Retinal dystrophy is inherited as a single autosomal recessive gene (*rdy*). Homozygotes have a progressive loss of the retinal photoreceptor cells and an overproduction of rhodopsin early in the disease process (LaVail *et al.*, 1972). A retinal degeneration reported in the Wag/Rij rat appears to be an expression of an autosomal dominant gene. End-stage lesions include disappearance of photoreceptor cells, migration of the pigment epithelium, and disorganization of remaining retinal layers. This retinal disease appears to mimic the pathogenesis of retinitis pigmentosa in humans (Lai *et al.*, 1980). Other reported developmental disorders of the eye include colobomas and cataracts.

Reports of congenital heart disease are rare. Among the few reports is one involving an inbred strain of Long-Evans rats (Fox, 1969). In this strain, approximately 25% of the neonates have interventricular septal defects. This malformation is believed to be of polygenic origin.

Unlike the mouse, there are few reported mutants that have disorders of the central nervous system. Recently, a partially inbred strain has been developed in which one subline has nearly a 50% incidence of hydrocephalus (Kohn *et al.*, 1981).

The mode of inheritance appears to be polygenic. The hydrocephalus is classified as communicating and the pathogenesis may be due to poorly developed veins in the periosteal and dural layers, and to underdeveloped pia-arachnoid cells.

2. Age-Related Diseases

Neoplastic and nonneoplastic lesions vary in type, onset, and prevalence due to the hereditary and environmental factors associated with a specific colony of laboratory rat. The rat is widely used in gerontological research. Those who use the rat must be clearly aware of the factors that can influence lesions and death in aged rats (Anver and Cohen, 1979; Burek, 1978). Neoplastic disease, a cause of death in many aged rats, has been discussed previously, and this section will summarize the more commonly seen nonneoplastic lesions in aging rats.

a. Chronic Progressive Nephropathy (CPN). Chronic progressive nephropathy is among the most commonly encountered lesions in the rat, particularly in the aged animal where it is a major life-limiting disease. Because of its complex nature, CPN has a large number of synonyms, often with inaccurate descriptive titles. Sequential development of lesions and factors that modify the course of CPN have been thoroughly studied, but the actual pathogenesis remains undetermined. Chronic progressive nephropathy occurs in most rats, but its rate of development is significantly influenced by numerous factors, including sex, genetics, age, diet, association with microflora, hormone treatment (particularly testosterone), and others. Male rats have an earlier onset and more rapid progression of CPN than females, and albino strains and stocks seem to be more predisposed. Diet is an extremely important predisposing factor, particularly the quality and quantity of dietary protein. Significant differences in severity of CPN can be observed among rats of the same sex, strain, age, and source when fed different diets for their lifetime. Axenic rats do not seem to develop significant CPN.

Rats with CPN develop progressively severe proteinuria, first with the selective excretion of only small molecules such as albumin. In late CPN there is nonselective excretion, and the electrophoretic pattern of urinary protein resembles that of serum. Proteinuria related to CPN should not be confused with the normal urinary excretion of α-globulin in male rats, probably of tubular origin. The histopathology of CPN is characterized as progressive basement membrane thickening of the glomerulus, Bowman's capsule, and proximal tubule. Basement membranes split and wrinkle as tubules become atrophic and collapse. Glomeruli enlarge, with mesangial deposition of protein and lipid, adhesion to Bowman's capsule, and segmental sclerosis. Tubular epithelium may contain granules of resorbed protein, which later is mixed with lipofuscin and hemosiderin. As glomerular protein leakage becomes more severe, tubules dilate and accumulate hyaline eosinophilic castes of protein within their lumina, resembling colloid. Tubular epithelium may undergo atrophy with collapse or dilatation. Interstitial fibrosis and leukocytic infiltration is frequently observed. Grossly, the kidneys become enlarged, pale with irregular surfaces and pigmented (Fig. 14). Cystic tubules can be observed. Hypoproteinemia, parathyroid hyperplasia with serum chemical changes indicative of hyperparathyroidism, and nephrotic syndrome are observed in rats with severe CPN (Barthold, 1979).

b. Nephrocalcinosis. This disease, which occurs more frequently in females, has been reported in numerous strains of rats. In most cases, affected rats have been fed purified diets (e.g., AIN-76). Renal lesions usually involve the corticomedullary junction and consist of mineral deposition in the lumina of the proximal tubules (Nguyen and Woodward, 1980).

c. Polyarteritis nodosa. This disease is of unknown etiology and affects muscular arteries, most commonly the mesenteric, pancreatic, and spermatic. The lesions may be either acute or chronic. In the acute stage, there is intimal and medial fibrinoid necrosis, focal thrombosis, disruption of the elastic

Fig. 14. Chronic progressive nephropathy in an aged rat. The kidneys are pale with irregular, pitted surfaces.

lamellae, and an infiltration of polymorphonuclear leukocytes and mononuclear cells. In the chronic stage, the arteries are nodular, tortuous, and thick-walled. Affected vessels frequently have aneurysms and thrombi. Both acute and chronic phases may be observed in the same artery in an animal.

d. Myocardial degeneration. Degeneration is a commonly observed lesion that occurs in most stocks and strains, with an onset at 12–18 months of age. It occurs more frequently in males. The lesions are usually microscopic but, in advanced stages, can appear grossly as grayish foci. The papillary muscles and their attachment sites in the wall of the left ventricle are the most frequent sites of the lesion (Anver and Cohen, 1979).

e. Radiculoneuropathy. Spinal nerve root degeneration has been reported in a number of rat stocks and strains. Lesion distribution may vary among strains; however, the cauda equina and ventral spinal nerve roots are commonly involved. Posterior paresis and paralysis have been associated with these lesions, but some suggest that these signs are associated with a separate degenerative process of the skeletal muscle (Anver and Cohen, 1979).

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