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Chapter 3

Biology and Diseases of Mice

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I. INTRODUCTION

A. Origin and History

The laboratory mouse is assigned to the genus *Mus,* subfamily Murinae, family Muridae, order Rodentia. Anatomical features of the molar teeth and cranial bones help differentiate it from other murids. The house mouse of North America and Europe, *Mus musculus,* is the species commonly used for biomedical research. Laboratory strains were usually derived from mice bred by mouse fanciers and their genomes are a mixture of *M. musculus musculus* (from eastern Europe) and *M. m. domesticus* (from western Europe). Since the mid-1980s, strains have been developed from Asian mice (*M. m. castaneus* from Thailand and *M. m. molossinus* from Japan) and from *M. spretus.*

The laboratory mouse was employed in comparative anatomical studies as early as the seventeenth century, but accelerated interest in biology during the nineteenth century, a renewed interest in Mendelian genetics, and the research requirement for a small, economical mammal that was easily housed and bred were instrumental in the development of the "modern" laboratory mouse. These studies have grown exponentially during the current century with the recognition of the power of the mouse for gene and comparative mapping and have made the laboratory mouse, in genetic terms, the most thoroughly characterized mammal on earth (Morse, 1979; Silver, 1995; Lyon *et al.,* 1996). The current ability to create highly sophisticated, genetically

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engineered mice by inserting transgenes or targeted mutations into endogenous genes has also made the laboratory mouse the most widely and heavily used experimental animal.

B. Genetics

Genetic mapping in mice began in the early 1900s. The first autosomal genes, albino and pink-eyed dilution, were linked in 1915 (Haldane *et al.,* 1915). Extensive linkage maps and an impressive array of inbred strains are now available to expedite sophisticated genetic research. Mice have 40 chromosomes that are differentiated by the size and patterns of transverse bands. The chromosomes are designated by Arabic numbers in order of decreasing size. During the 1970s, chromosome rearrangements were used to assign known genetic linkage groups—identified by Roman numerals—to specific chromosomes and for determining locus order with respect to the centromere. Genes can now be located physically on chromosomes by fluorescent *in situ* hybridization (FISH), and the Genome Initiative programs are fostering development of molecular maps of mouse chromosomes. The sequence of the mouse genome is expected to be completed by 2003 (Battey *et al.,* 1999).

Inbred mice are valuable for research in virtually all fields of biomedical research, such as immunology, oncology, cardiovascular disease, metabolic disease, microbiology, biochemistry, pharmacology, physiology, anatomy, developmental biology, and radiobiology. Mice carrying spontaneous or induced mutations and strains susceptible to specific diseases provide a wide variety of mouse model systems for basic research, as well as models for biomedical research to understand specific human disorders. For example, several spontaneous mutations in genes affecting pituitary function or producing hormones provide mutant models for human dwarfing conditions. Targeted mutations in the low-density lipoprotein receptor and apolipoprotein genes provide model systems for studying cardiovascular disorders. Mice of the NOD (nonobese diabetic) strain provide a model for human insulin-dependent diabetes. Mice also provide reagents for basic research. For example, inbred histocompatible strains are used extensively as donors of plasma cell tumors to immortalize cell lines (hybridomas) that secrete highly uniform, monospecific immunoglobulins *in vitro,* theoretically in unlimited quantities. This technology has made a full range of functional mouse antibody molecules available for study. Development of quantitative trait loci (QTL) methodology for mapping genes and the similarity between mouse and human genomes have made the mouse invaluable for identifying genes and underlying complex traits that are inherent to the most common human genetic diseases (Darvasi, 1998; Frankel, 1995).

One of the most thoroughly studied genetic systems of the mouse is the *histocompatibility complex.* Histocompatibility (*H*) loci control expression of cell surface molecules that modulate major immunological phenomena, such as the recognition of foreign tissue. For example, the time, onset, and speed of skin graft rejection are controlled by two groups of *H* loci. The major group is located in the major histocompatibility complex (MHC, *H2*) on chromosome 17. These genes cause rapid rejection (10–20 days) of grafts that display foreign *H2* antigens. Minor *H* loci groups are scattered throughout the genome and are responsible for delayed graft rejection. Genes associated with the *H2* complex also control other immunological functions, such as cell–cell interactions in primary immune responses and the level of response to a given antigen. Immune-mediated responses to infectious agents such as viruses and complement activity are influenced directly or indirectly by the *H2* complex. The most recent comprehensive review of the *H2* complex is by Klein (1986). Because information about this subject is being published so frequently, the reader is advised to consult bibliographic indexes such as MEDLINE for recent updates. Non-MHC or minor histocompatibility systems also are under active study (Roopenian and Simpson, 2000).

C. Breeding Systems and Nomenclature

1. Breeding Systems

Laboratory mice are identified by strain and by breeding system. A genealogy of most inbred strains is presented in the Mouse Genome Database <http://www.informatics.jax.org>. Table I summarizes nine breeding systems. Each requires technical skill and a firm understanding of mammalian genetics. *Inbred* strains were developed first in 1909 by Clarence Cook Little and offer a high degree of genetic uniformity. Mice within an inbred strain, for practical purposes, are genetically identical to other mice of the same strain and sex. They are defined as being produced by brother–sister matings for more than 20 generations. In fact, a strain should not be considered completely inbred until after 40 generations of sibling matings. Inbred strains are valuable because experimental results are reproducible with relatively small sample sizes. They are useful for genetic mapping because they are genetically well characterized, and allelic combinations can be predetermined for linkage crosses. Wild-derived inbred strains of *Mus musculus castaneus* and *M. spretus* are used extensively for mapping because of the large number of polymorphic differences from standard inbred laboratory mice. F_1 *hybrid* mice, produced by mating mice of two inbred strains, also are genetically identical to each other and may offer a more robust animal for some studies. For example, inbred strains may differ in behavior and learning abilities, whereas hybrid mice are less likely to have learning deficits or behavioral anomalies. Mutant inbred strains carry spontaneous, targeted, or induced mutations, transgenes, or chromosome aberrations. The genetic backgrounds of such strains are homogeneous like those of regular inbred strains, but some (or all) mice of the strain carry the mutation (or chromosome aber-

Table I

Kinds of Mice Used in Research[a]

Definition of breeding system	Perpetuation of breeding system	Reference
Random bred stock: Random mating within a large, heterogeneous population	Continue random mating, selection pairs with random numbers method	Poiley (1960) Kimura and Crow (1963)
Inbred strain: Brother–sister mating for more than 20 generations	Continue brother–sister mating	Green (1981a)
F_1 hybrids: Mice from crosses between inbred strains	Cannot be perpetuated	Green (1981a)
Segregating inbred strain: Brother–sister matings system for more than 20 generations with heterozygosity for the mutations forced by (1) backcrossing, (2) intercrossing, (3) crossing and intercrossing, or (4) backcrossing and intercrossing	Continue brother–sister mating with heterozygosity forced by one of the four methods at left or with homozygosity forced by intercrossing homozygotes	Green (1981a)
Coisogenic inbred strains: Occurrence of a mutation within a strain	Perpetuate the mutation by (1) brother–sister mating within strain of origin, (2) backcross or cross–intercross system with strain of origin as parent strain, (3) brother–sister mating with heterozygosity forced by back- or intercrosses, or (4) brother–sister mating between homozygotes	Flaherty (1981) Green (1981a)
Congenic inbred strains: (A) Repeated backcross of mutation-bearing mice for 10 or more generations or (B) cross–intercross system for the equivalent of 20 or more cycles with an inbred parent strain	Perpetuate the transferred mutation by (2), (3), or (4) above. (1) may be used after 10–12 generations of backcrossing with periodic backcrosses to background strain	Flaherty (1981) Green (1981a)
Recombinant inbred strains: Brother–sister matings for >20 generations after crossing two inbred strains and their F_1 to obtain and F_2	Continue brother–sister matings	Bailey (1971)
Recombinant congenic strains: Same as above except one or more backcrosses of F_1 to one parent strain before beginning brother–sister matings	Continue brother–sister matings	Demant and Hart (1986)
Advanced intercross lines: Nonsibling matings from an F_2 of a cross between two inbred strains	Continue nonsibling matings	

[a]Modified from Green (1981a).

ration). In segregating inbred mutant strains, mutant mice differ from their nonmutant siblings only by the mutation. Therefore, littermates can serve as controls in experiments. In homozygous mutant strains, controls must come from the same or a closely related nonmutant inbred strain. For example, C57BL/6J mice provide controls for the homozygous mutant C57BL/6J-*m/m* strain. A strain is said to be *coisogenic* if the mutation of interest occurred in that strain. A strain is *congenic* if the mutation or gene of interest was transferred from another strain or stock by repeated backcrossing. Detailed descriptions and diagrams of mating schemes have been provided by Green (1981a,b), and additional contemporary information on mouse genetics and breeding can be found by consulting Table II.

In contrast to inbred mice, *random bred, or outbred,* mice are genetically heterogeneous and are often produced by breeding systems that intentionally minimize inbreeding. Outbred mice may be used when high genetic heterogeneity is desired or for experiments requiring large numbers of mice. However, it is preferable to ensure genetic heterogeneity by intercrossing multiple inbred strains to achieve heterogeneity with known genetic input. Individual random bred mice within a colony may differ in coat color, histocompatibility loci, enzyme and DNA polymorphisms, and other characteristics. Random breeding requires the statistically random selection of breeders by using a random numbers table or computer program. Random breeding, or outbreeding, can be achieved only in a large colony. A small breeding population or passage through the genetic "bottleneck" of rederivation to improve health status will reduce genetic heterogeneity and lead eventually to some degree of inbreeding. In fact, supposedly "random bred" stocks are often genetically quite homogeneous. In a population of 25 breeding pairs, for example, heterozygosity will decrease at 1% per generation with standard randomization techniques. A random breeding program that is easy to manage is the circular pair mating system, in which each pair is mated only once. Conceptually, cages are visualized in a circle, and each cage contains one breeding pair in the nth generation. Another "circular" set of cages serves as the breeding nucleus for the $n + 1$ generation. Each mated pair in the nth generation contributes one female and one male to the $n + 1$ generation. Random breeding is accomplished by assigning the female and male derived from each nth generation cage to different cages in the $n + 1$ generation.

Table II

Databases and Websites for Information about Mice

Internet resource	Web address
Comprehensive database sites and mouse sources	
Mouse Genome Database (MGD)	http://www.informatics.jax.org/
JAX Mice	http://jaxmice.jax.org/index.shtml
MRC Mammalian Genetics Unit, Harwell, United Kingdom	http://www.mgu.har.mrc.ac.uk/
The Whole Mouse Catalog	http://www.rodentia.com/wmc/
ORNL Mutant Mouse Database	http://bio.lsd.ornl.gov/mouse/
Genetically engineered mouse sites and sources	
Induced Mutant Resource	http://lena.jax.org/resources/documents/imr/
TBASE	http://tbase.jax.org/
European Mouse Mutant Archive (EMMA)	http://www.emma.rm.cnr.it/
BioMedNet Mouse Knockout and Mutation Database	http://research.n.com/mkmd
Cre Transgenic and Floxed Gene Databases	http://www.mshri.on.ca/nagy/cre.htm
University of California Resource of Gene Trap Insertions	http://socrates.berkeley.edu/~skarnes/resource.html
Database of Gene Knockouts	http://www.bioscience.org/knockout/knochome.htm
The Big Blue Web Site	http://eden.ceh.uvic.ca/bigblue.htm
The Mouse Brain Library	http://www.nervenet.org/mbl/mbl.html
Mouse biology	
Mouse Tumor Biology Database (MTB)	http://tumor.informatics.jax.org/cancer links.html
The Mammary Transgene Database	http://bcm.tmc.edu/ermb/mtdb/mtdb.html
Gene Expression Database (GXD)	http://www.informatics.jax.org/
NetVet and the Electronic Zoo	http://netvet.wustl.edu/vet.htm
The Dysmorphic Human–Mouse Homology Database (DHMHD)	http://www.hgmp.mrc.ac.uk/dhmhd/dysmorph.html
The Mouse Atlas and Gene Expression Database Project	http://genex.hgu.mrc.ac.uk/
UCD Medpath Transgenic Mouse Searcher 2.0	http://www-mp.ucdavis.edu/personaltgmouse1.html
Mouse 2-D PAGE Database	http://biosun.biobase.dk/~pdi/jecelis/mouse_data_select.html
Body map	
Human and Mouse Gene Expression DB	http://bodymap.ims.u-tokyo.ac.jp/
UNSW Embryology Mouse Development	http://anatomy.med.unsw.edu.au/cbl/embryo/otheremb/mouse.htm
Dynamic [Embryonic] Development	http://www.acs.ucalgary.ca/~browder/mice.html
Zygote: A Developmental Biology Website	http://zygote.swarthmore.edu/info.html
Mouse genomics	
Mouse Nomenclature Guidelines and Locus Symbol Registry	http://www.informatics.jax.org/mgihome/nomen/
Trans-NIH Mouse Initiative	http://www.nih.gov/science/models/mouse/
Gene Dictionary of the Mouse Genome	http://www.nervenet.org/main/dictionary.html
Genetic and Physical Maps of the Mouse Genome	http://www-genome.wi.mit.edu/cgi-bin/mouse/index
Mouse Backcross Service (U.K. HGMP Resource Centre)	http://www.hgmp.mrc.ac.uk/goneaway/mbx.html
The Jackson Laboratory Mapping Panels	http://www.jax.org/resources/documents/cmdata/
WashU GSC Mouse EST Project	http://genome.wustl.edu/est/mouse_esthmpg.html
Japanese Animal Genome Database	http://ws4.niai.affrc.go.jp/
NCBI LocusLink	http://www.ncbi.nlm.nih.gov/focuslink/
UniGene Mouse Sequences Collection	http://www.ncbi.nlm.nih.gov/unigene/mm.home.html
TIGR Mouse Gene Index	http://www.tigr.org/tdb/mgi/index.html
NIA/NIH Mouse Genomics Home Page	http://lgsun.grc.nia.nih.gov/
WICGR Mouse RH Map Home Page	http://www-genome.wi.mit.edu/mouse_rh/index.html
Mammalian Genetics Laboratory, National Institute of Genetics (Japan)	http://www.shigen.nig.ac.jp/mouse/mouse.default.html
Care and use	
Guidelines for Ethical Conduct in the Care and Use of Animals	http://www.apa.org/science/anguide.html
The Ethics of Using Transgenic Animals	http://oslovet.veths.no/transgenics/references.html
Institute for Laboratory Animal Research	http://www4.nationalacademies.org/cls/ilarhome.nsf
Laboratory Registration Code Database	http://www4.nas.edu/cls/afr.nsf/labcodesearch?openform
Research Genetics, Genomic Tools	http://www.resgen.com/index.php3
General	
American Fancy Rat and Mouse Association	http://www.afrma.org/

Recombinant inbred (RI) strains are sets of inbred strains developed by single-pair random matings of mice from an F_2 generation created by crossing mice of two inbred strains. Lines are propagated by brother–sister matings for more than 20 generations to obtain homozygosity. Recombinant inbred strains may take as long as 7 years to produce. RI strains are valuable for mapping phenotypic or quantitative traits that differ between the progenitor strains. Because each line is inbred, genotyping and phenotyping data are cumulative. RI sets are especially valuable for controlling for environmental variability in a trait, because several genetically identical mice from each line in a set can be typed to score the line for a trait (Bailey, 1971; Taylor, 1996). *Recombinant congenic* strains are sets of inbred strains derived in a manner similar to that for RI sets, except that one or more backcrosses to one parental strain (designated the background strain) are made after the F_1 generation, before inbreeding is begun. The other parental strain is designated as the donor strain. The proportion of background and donor genomes is determined by the number of backcrosses preceding inbreeding (Demant and Hart, 1986). Advanced intercross Lines (AILs) are a third type of RI line. AILs are made by producing an F_2 generation between two inbred strains and then, in each subsequent generation, intercrossing mice but avoiding sibling matings. The purpose is to increase the possibility of recombination between tightly linked genes. For further information on this topic, consult Genetic Guidelines, Mouse Genome Database <http://www.informatics.jax.org>.

2. Nomenclature

There are currently more than 1000 separate outbred stocks and inbred strains, some with multiple sublines. In addition, there are thousands of mutant strains. Therefore it is critical that strain or stock designations be complete and accurate to avoid semantic and genetic confusion. As an example of subline variation that makes precise nomenclature important, CBA/J carries the gene for retinal degeneration, while the CBA/CaJ subline does not.

Specific nomenclatures have been developed for inbred and noninbred strains and stocks. Strains are designated by a series of letters and/or numbers, which frequently provide a shorthand description of the origin and history of the strain (Table III). For example, the inbred strain C57BL/6J originated from female 57 at the Cold Spring Harbor Laboratory (C), was the black (BL) line from this female, and is subline number 6. Sublines of an inbred strain are designated using Laboratory Registration Codes (Lab Codes), unique–2- to 4-letter codes that may be obtained from a central registry maintained at the Institute for Laboratory Animal Research in Washington, D.C. <http://www4.nationalacademies.org/cls/ilarhome.nsf>. The J in C57BL/6J means it is the subline maintained at the Jackson Laboratory (J). A new type of strain designation has been created for new inbred strains made by intercrossing mice of two existing inbred strains. This is essential because many engineered mutations are made in 129-derived embryonic stem cells, recovered in C57BL/6-129 chimeras, and then maintained by brother–sister matings after the first generation. For example, an inbred strain derived by sibling matings from a C57BL/6 × 129 chimera is designated B6129. A noninbred stock that meets specific criteria is designated by placing the Lab Code before the stock symbol, separated by a full colon. For example, Hsd:ICR designates an ICR outbred stock maintained by Harlan ("International standardized nomenclature," 1972). Specific designations also distinguish coisogenic, congenic, segregating inbred, and various RI strains. The type of strain or stock often can be recognized from the correct symbol. For example, BXD-1/Ty is line 1 in a set of RI strains derived from a C57BL/6J (B) female mated to a DBA/2J (D) male and made by Taylor (Ty).

Mutant genes are designated by a brief abbreviation for the mutation (e.g., *shi* for shiverer). When a mutant gene is cloned, the symbol for the parent gene is used and the mutant allele is designated in superscript. For example, Mbp^{shi} is the shiverer mutant allele in the myelin basic protein gene. Nomenclature for genetically engineered mice can be complex and may eventually require simplification. Currently, a *transgenic strain* is designated by a symbol for the strain followed by a symbol for the transgene. Transgene symbols take the form Tg(YYYYY) #Zzz, where Tg is the transgene, (YYYYY) is a brief description of the inserted DNA (such as a gene symbol), # is the assigned number in the series of events generated using a given construct, and Zzz is the Lab Code. When a transgene causes an insertional mutation in an endogenous gene, the mutant allele of the gene is designated by using the gene symbol and an abbreviation for the transgene as a superscript (e.g., $in^{Tg/Zzz}$). A targeted mutation, or knockout, is designated by the mutated gene with the identification of the mutational event as a superscript. For example, $Cftr^{fm1\ Unc}$ is the first mutation in the cystic fibrosis transmembrane regulator gene created at the University of North Carolina. A gene replacement, or knockin, uses similar nomenclature; $Myf5^{Myod}$ indicates that the *Myf5* gene was replaced by the *Myod* gene.

The International Committee on Standardized Genetic Nomenclature for Mice, established in the early 1950s, is responsible for genetic nomenclature rules. The rules are available online at the Mouse Genome Database (MGD) website <http://www.informatics.jax.org>. They are published periodically in print copy, the most recent being in Davisson (1996). The committee also maintains a list of inbred strains at the MGD site. The reader is refered to Chapters 27 and 28 for further discussion of nomenclature and to Table II for selected databases and websites relevant to mouse genetics and biology that are available as of this writing.

Table III

Examples of Mouse Strain Nomenclature

Strain name	Definition
DBA/2J	Inbred strain named for its characteristic coat color genes (using their original gene symbols), dilute (*d*), brown (*b*), and nonagouti (*a*); it is the second of two sublines separated before 20 generations of brother × sister breeding and is the subline maintained at the Jackson Laboratory (J)
C3H/HeSn-*ash*/+	Coisogenic segregating inbred mutant strain carrying the ashen (*ash*) mutation, which arose on C3H/HeSn
C57BL/6J-*Tyr*$^{c\text{-}2J}$/+	Coisogenic segregating inbred mutant strain carrying the albino 2J mutant allele of the cloned tyrosinase gene (*tyr*)
AEJ/GnJ-*a*^{e}/*A*$^{w\text{-}J}$	Inbred strain segregating for two alleles at the agouti gene
AKR.B6-*H2b*	Congenic inbred strain in which the *b* haplotype at the *H2* complex was transferred from C57BL/6J (B6) to the AKR background
B6.CBA-*D4Mit25-D4Mit80*	Congenic strain in which the chromosomal segment between *D4Mit25* and *D4Mit80* was transferred from CBA to B6
B6.Cg *m Lepr*^{db}/++	Congenic inbred strain in which the linked mutant genes misty (*m*) and diabetes (*Lepr*^{db}) were transferred from multiple, mixed, or unknown genetic backgrounds to B6 and are carried in coupling, i.e., on the same chromosome
B6.Cg-*m* +/+ *Lepr*^{db}	Congenic inbred strain in which the *m* and *Lepr*^{db} mutations are carried in repulsion
BXD-1/Ty	Recombinant inbred (RI) strain number 1 in a set of RI strains derived from a C57BL/6J (B) female mated to a DBA/2J (D) male and made by Taylor (Ty)
CcS1	Recombinant congenic (RC) strain number 1 in a set made by crossing the BALB/c (C) and STS (S) strains, backcrossing 1 or 2 times to BALB/c and then inbreeding as with RI strains
CcS1(N4)	Recombinant congenic (RC) strain number 1 in a set made by crossing the BALB/c (C) and STS (S) strains, backcrossing N4 times to BALB/c and then inbreeding as with RI strains
B.A-Chr 1	Chromosome substitution (CSS) or consomic strain in which Chr 1 from A/J has been transferred to the B6 background
C57BL/6J-mt$^{BALB/c}$	Conplastic strain with the nuclear genome of C57BL/6J, and the cytoplasmic genome of BALB/c, developed by crossing male C57BL/6J mice with BALB/c females, followed by repeated backcrossing of female offspring to male C57BL/6J
B6;129-*Cftr*^{tm1Unc}	First targeted mutation of the cystic fibrosis transmembrane regulator gene created at the University of North Carolina, Unc, and carried on a mixed B6 and 129 background
B6.129-*Myf5*^{Myod}	Congenic strain carrying a replacement or "knockin" in which the *Myf5* gene was replaced with the *Myod* gene in 129 ES cells and backcrossed onto the B6 genetic background
FVB/N-TgN(MBP) 1Xxx	Transgene in which the human myelin basic protein (*MBP*) gene is inserted into the genome of the National Institutes of Health (N) subline of the FVB strain originally maintained at the National Institutes of Health
FVB/N-*m*^{Tg1Zzz}	Insertional mutation caused by the *Tg1Zzz* transgene made on the FVB/N genetic background
B6C3F1	F_1 hybrid made by crossing a C57BL/6 female to a C3H male
B6EiC3-Ts65Dn	Strain maintained by backcrossing mice with the Ts65Dn chromosome aberration to F_1 hybrid mice made by crossing females of the Eicher (Ei) subline of C57BL/6 × C3H; note that these mice are not true F_1 hybrids, and the F_1 designation is omitted
Hsd:ICR	ICR outbred stock maintained at Harlan (Hsd)
Pri:B6,D2-G#	Advanced intercross line (AIL) created at Princeton (Pri) from the inbred strains C57BL/6 × DBA/2; AIL are made similar to RI strains except mice are intercrossed, avoiding sibling matings, to increase the possibility of tightly linked genes recombining

D. Housing and Husbandry

1. Housing

Housing (and husbandry) for mice are often guided by microbiological requirements. A colony can be maintained in a "conventional" environment or behind a barrier where the mice are protected from specific microorganisms. Examples of barrier housing include positive pressure isolators and mass airflow racks that provide sterile air through high-efficiency particulate air (HEPA) filters or individually ventilated caging. The integrity of the microenvironment is maintained by servicing and changing cages in specifically designed hoods.

Mouse cages vary in design, size, and composition. The popular shoebox cage used for housing and breeding mice is usually made of polycarbonate, polypropylene, or polystyrene plastic (in order of decreasing cost and durability). Mice are sometimes housed in suspended cages with open-mesh bottoms that allow excrement to fall through to a collecting pan. Suspended caging is rarely used for breeding because neonatal thermoregulation is difficult to maintain without nesting material. Cage lids should be stainless steel to facilitate cleaning and inhibit rust. Cages should keep animals dry and clean, maintain a comfortable ambient temperature, allow freedom of movement and normal postural adjustments, avoid unnecessary physical restraints, provide convenient access to feed and water, and prevent overcrowding.

Solid-bottom cages should contain sanitary bedding, such as wood chips or ground corncob. Criteria for selecting bedding

Table IV

Desirable Criteria for Rodent Contact Bedding[a]

Moisture absorbent
Dust-free
Does not promote microbial growth
Nonstaining
Atraumatic
Ammonia binding
Sterilizable
Deleterious products not formed as a result of sterilization
Easily stored
Uniform from batch to batch
No microbial or chemical contamination
Nonpalatable
Nonallergenic
Nontoxic
Non-enzyme-inducing
Nestable
Readily available
Inexpensive
Chemically stable during use
Animal behavior is not adversely affected

[a]Modified from Kraft (1980).

Table V

Tests of Bedding Quality[a]

Chemical properties
Pesticides and polychlorinated compounds
Mycotoxins
Nitrosamines
Detergent residues
Ether-extractable substances
Heavy metals
Physical properties
Particle uniformity
Absorbtivity
Ammonia evolution
Visible trauma and irritant potential
Microbiological properties
Standard plate count
Yeasts and molds
Coliforms and *Salmonella*
Pseudomonas

[a]Modified from Kraft (1980).

vary with experimental and husbandry needs (Table IV). It is preferable to autoclave bedding prior to use, but if this is not convenient, the bedding should be used only after its origin and microbial content have been evaluated (Table V). Several caging systems with tops containing filters are routinely in use in many academic settings. Their use has been popularized because of evidence that they substantially reduce or prevent airborne transmission of microbial agents (Lipman *et al.,* 1993) and minimize caretaker exposure to allergens (Reeb-Whitaker *et al.,* 1999). However, this type of caging employs passive air exchange and is prone to accumulate CO_2 and NH_3, thereby requiring frequent sanitation and bedding changes (see Chapter 29). The recent large-scale introduction of individually ventilated cages obviate, to a large extent, the elevated levels of noxious gases. However, purchase of these systems requires substantial expense (see Chapter 21).

2. Husbandry

Nutrient requirements for the mouse are influenced by genetic background, disease status, pregnancy, and environment. The best current estimate of nutritional requirements is shown in Table VI. Nutritional requirements for laboratory mice are also published periodically by the National Research Council and have been reviewed by Knapka and coworkers (Knapka, 1983; Knapka *et al.,* 1974). Feed intake and weight gain data are used to estimate the nutritional needs of a particular stock or strain. Mice consume about 3–5 gm of feed per day after weaning and maintain this intake throughout life. Outbred mice tend to gain weight faster than inbred mice and are heavier at maturity (Figs. 1 and 2).

Diet is often neglected as a variable in animal-related research. Diet can influence responses to drugs, chemicals, or other factors and lead to biased research results. Therefore, diet must provide a balance of essential nutrients, and contaminants must be kept to a minimum (see also Chapter 29). Natural-product commercial diets for mice are satisfactory for breeding and maintenance. Fresh produce, grains, fish meal, or other supplements may expose colonies to pathogenic bacteria or harmful chemicals and should be avoided.

Mice should have continuous access to potable water even if a high-moisture diet is fed. Water is needed for lubrication of dry food and for hydration. Adult mice drink 6–7 ml of water per day. Decreased water intake will decrease food consumption. Water imbalance may occur during disease, because sick mice commonly drink very little water. Therefore, it may be unsuitable to administer medicine orally to affected mice. However, antimicrobials can be administered in the drinking water prophylactically, a measure used commonly to prevent infection in immunodeficient mice.

II. BIOLOGY

A. Physiology and Anatomy

Unless otherwise indicated the information in this section is from Cook (1983) and Kaplan *et al.* (1983). Normative data on the mouse are presented in Table VII, and clinical chemistry reference ranges are summarized in Table VIII.

Table VI

Nutrient Requirements of Mice[a]

Nutrient	Concentration in diet (%)
Protein (as crude protein)	20–25
Fat[b]	5–12
Fiber	2.5
Carbohydrate	45–60

Estimated Dietary Amino Acid Requirement

Amino acid	Natural-ingredient, open-formula diet (%)[c]	Purified diet (%)[d]
Arginine	0.3	—
Histidine	0.2	—
Tyrosine	—	0.12
Isoleucine	0.4	0.2
Leucine	0.7	0.25
Lysine	0.4	0.15
Methionine	0.5	0.3
Phenylalanine	0.4	0.25
Threonine	0.2	0.22
Tryptophan	0.1	0.05
Valine	0.5	0.3

Mineral and Vitamin Concentrations of Adequate Mouse Diets

Mineral	Natural-ingredient, open-formula diet[e]	Purified diet[f]	Purified diet[g]	Chemically defined diet[h]
Calcium (%)	1.23	0.52	0.81	0.57
Chloride (%)	—	0.16	—	1.03
Magnesium (%)	0.18	0.05	0.073	0.142
Phosphorus (%)	0.99	0.4	0.42	0.57
Potassium (%)	0.85	0.36	0.89	0.40
Sodium (%)	0.36	0.1	0.39	0.38
Sulfur (%)	—	—	—	0.0023
Chromium (mg/kg)	—	2.0	1.9	4.0
Cobalt (mg/kg)	0.7	—	—	0.2
Copper (mg/kg)	16.1	6.0	4.5	12.9
Fluoride (mg/kg)	—	—	—	2.3
Iodine (mg/kg)	1.9	0.2	36.0	3.8
Iron (mg/kg)	255.50	35.0	299.0	47.6
Manganese (mg/kg)	104.0	54.0	50.0	95.2
Molybdenum (mg/kg)	—	—	—	1.55
Selenium (mg/kg)	—	0.1	—	0.076
Vanadium (mg/kg)	—	—	—	0.25
Zinc (mg/kg)	50.3	30.0	31.0	38.0

Vitamin	Natural-ingredient, open-formula diet[e]	Purified diet[f]	Purified diet[g]	Chemically defined diet[h]
A (IU/kg)	15,000	4000	1100	1730
B_6 (mg/kg)	10	7	22.5	6.0
B_{12} (mg/kg)	0.03	0.01	0.023	0.58
D (IU/kg)	5000	1000	1100	1.71
E (IU/kg)	37	50	32	1514
K_1 equiv. (mg/kg)	3	0.05	18	10.7
Biotin (mg/kg)	0.2	0.2	0.2	1
Choline (mg/kg)	2009	1000	750	2375
Folacin (mg/kg)	4	2	0.45	1.43
Inositol (mg/kg)	—	—	—	248
Niacin (mg/kg)	82	30	22.5	35.6
Calcium pantothenate (mg/kg)	21	16	37.5	47.5
Riboflavin (mg/kg)	8	6	7.5	7.1
Thiamin (mg/kg)	17	6	22.5	4.8

[a] Modified from Knapka (1983).
[b] Linoleic acid: 0.6% is adequate.
[c] John and Bell (1976).
[d] Theuer (1971).
[e] Knapka *et al.* (1974).
[f] AIN (1977).
[g] Hurley and Bell (1974).
[h] Pleasants *et al.* (1973).

1. Temperature and Water Regulation

Mice have a relatively large surface area per gram of body weight. This results in dramatic physiologic changes in response to fluctuations in the ambient temperature (T_A). The mouse responds to cold exposure, for example, by nonshivering thermogenesis. A resting mouse acclimated to cold can generate heat equivalent to about triple the basal metabolic rate, a change that is greater than for any other animal. A mouse must generate about 46 kcal/m^2 per 24 hr to maintain body temperature for each 1°C drop in T_A below the thermoneutral zone.

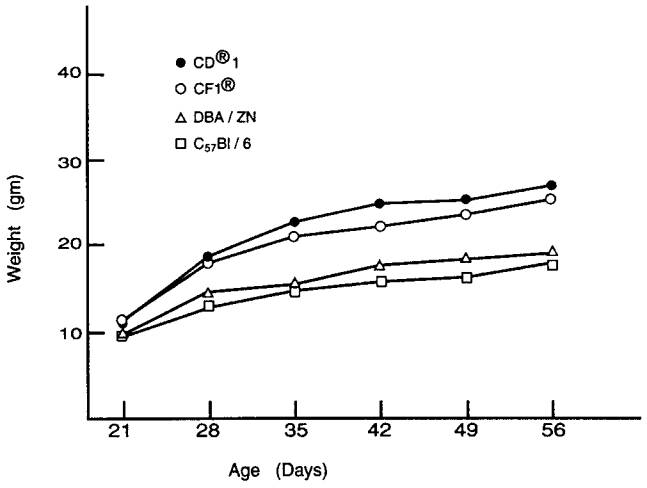

Fig. 1. Growth comparison: female outbred (CD1 and CF1) and inbred mice. (Courtesy of Charles River Breeding Laboratories.)

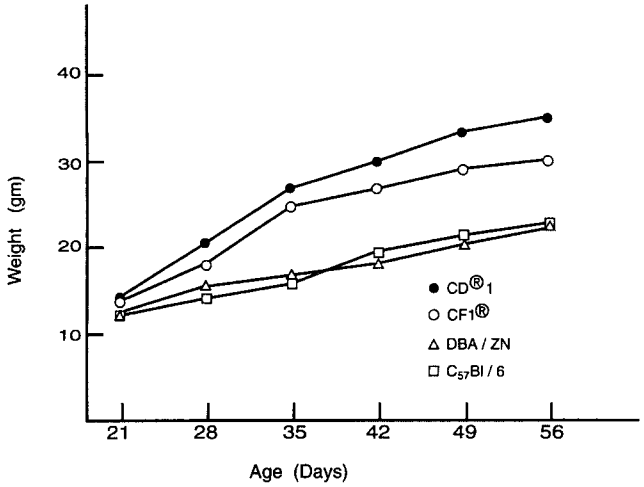

Fig. 2. Growth comparison: male outbred (CD1 and CF1) and inbred mice. (Courtesy of Charles River Breeding Laboratories.)

Mice cannot tolerate nocturnal cooling as well as larger animals that have a greater heat sink. Therefore, it is not advisable to conserve energy in animal quarters at night by lowering thermostats.

Because of its great ratio of evaporative surface to body mass, the mouse has a greater sensitivity than most mammals to water loss. Its biological half-time for turnover of water (1.1 days) is more rapid than for larger mammals. Water conservation is enhanced by cooling of expired air in the nasal passages and by highly efficient concentration of urine.

The conservation of water can preempt thermal stability. If the mouse had to depend on the evaporation of body water to prevent elevations of body temperature, it would go into shock from dehydration. The mouse has no sweat glands, it cannot pant, and its ability to salivate is severely limited. Mice can partially compensate for changes in T_A increases from 20° to 35°C. It adapts to moderate but persistent increases in environmental temperature by a persistent increase in body temperature, a persistent decrease in metabolic rate, and increased blood flow to the ears to increase heat loss. Its primary means of cooling in the wild is behavioral—retreat into a burrow. In the confinement of a cage, truck, or plane, mice do not survive well in heat and begin to die at an ambient temperature of 37°C or higher. Thus, the mouse is not a true endotherm. In fact, the neonatal mouse is ectothermic and does not have well-developed temperature control before 20 days of age.

The thermoneutral zone for mice varies with strain and with conditioning but is about 29.6°–30.5°C, narrower than that of any other mammal thus far measured. Thermoneutrality should not be equated with comfort or physiological economy. There are repeated studies to show that mice in a T_A range of 21°–25°C grow faster, have larger litters, and have more viable pups than those maintained in the thermoneutral zone.

Table VII

Normative Data for the Mouse

Adult weight	
Male	20–40 gm
Female	18–35 gm
Life span	
Usual	1–3 years
Maximum reported	4 years
Surface area	0.03–0.06 cm^2
Chromosome number (diploid)	40
Water consumption	6.7 ml/8 weeks age
Food consumption	5.0 gm/8 weeks age
Body temperature	98.8°–99.3°F (37°–37.2°C)
Puberty	
Male	28–49 days
Female	28–49 days
Breeding season	None
Gestation	19–21 days
Litter size	4–12 pups
Birth weight	1.0–1.5 gm
Eyes open	12–13 days
Weaning	21 days
Heart rate	310–840 beats/min
Blood pressure	
Systolic	133–160 mm Hg
Diastolic	102–110 mm Hg
Blood volume	
Plasma	3.15 ml/100 gm
Whole blood	5.85 ml/100 gm
Respiration frequency	163/min
Tidal volume	0.18 (0.09–0.38) ml
Minute volume	24 (11–36) ml/min
Stroke volume	1.3–2.0 ml/beat
Plasma	
pH	7.2–7.4
CO_2	21.9 mEq/L
CO_2 pressure	40 ± 5.4 mm Hg
Leukocyte count	
Total	8.4 (5.1–11.6) × $10^3/\mu l$
Neutrophils	17.9 (6.7–37.2)%
Lymphocytes	69 (63–75)%
Monocytes	1.2 (0.7–2.6)%
Eosinophils	2.1 (0.9–3.8)%
Basophils	0.5 (0–1.5)%
Platelets	600 (100–1000) × $10^3/\mu l$
Packed cell volume	44 (42–44)%
Red blood cells	8.7–10.5 × $10^8/mm^3$
Hemoglobin	13.4 (12.2–16.2) gm/dl
Maximum volume of single bleeding	5 ml/kg
Clotting time	2–10 min
PTT	55–110 sec
Prothrombin time	7–19 sec

2. Respiratory System

The respiratory tract has three main portions: the anterior respiratory tract consists of nostrils, nasal cavities, and nasopharynx; the intermediate section consists of larynx, trachea, and

Table VIII

Clinical Chemistry Reference Ranges for Adult Mice[a]

Analyte	Units	CD-1 M	CD-1 F	C57BL/6 M	C57BL/6 F	BALB/cBy M	BALB/cBy F
Serum							
Glucose	mg/dl	112 ± 38.1	97 ± 39.9	121.7 ± 33.2	134.4 ± 20.3	171.6 ± 57.2	174.9 ± 31.0
Urea nitrogen	mg/dl	38 ± 20.1	37 ± 16	32.7 ± 3.5	23.6 ± 5.3		
Creatinine	mg/dl	1.10 ± 0.45		0.50 ± 0.08	0.84 ± 0.298	0.43 ± 0.14	0.45 ± 0.07
Sodium	mEq/liter	166 ± 8.6	166 ± 4.1	166.7 ± 8.9	160.8 ± 4.40	157.8 ± 5.7	157 ± 6.70
Potassium	mEq/liter	8.0 ± 0.85	7.8 ± 0.75				
Chloride	mEq/liter	125 ± 7.2	130 ± 3.9				
Calcium	mg/dl	8.90 ± 2.06	10.30 ± 1.58			8.10 ± 0.80	
Phosphorus	mg/dl	8.30 ± 1.46	8.00 ± 1.85			5.95 ± 0.63	
Magnesium	mg/dl	3.11 ± 0.37	1.38 ± 0.28				
Iron	µg/dl	474 ± 44	473 ± 16				
Alanine aminotransferase	IU/liter	99 ± 86.3	49 ± 22.6	41.4 ± 16.4	29.3 ± 7.1		
Aspartate aminotransferase	IU/liter	196 ± 132.6	128 ± 60.6	99.5 ± 33.4	73.6 ± 15.3		
Alkaline phosphatase	IU/liter	39 ± 25.7	51 ± 27.3	59 ± 11.4	118 ± 15.9		
Lactate dehydrogenase	IU/liter					378 ± 269	
Protein, total	g/liter	44 ± 11.0	48 ± 8.5	53.9 ± 7.5	63.5 ± 8.8	55.7 ± 8.9	54.6 ± 8.3
Albumin	g/liter			36.7 ± 5.2	46.4 ± 7.0	31.7 ± 4.7	39.3 ± 5.4
Cholesterol	mg/dl	114 ± 56.3	72 ± 20.1	94.8 ± 16.9	92 ± 15.9	150.4 ± 29.9	118.2 ± 36.1
Triglycerides	mg/dl	91 ± 58.5	53 ± 23.6	97 ± 21.1	78 ± 12.2		
Bilirubin	mg/dl	0.4 ± 0.2	0.5 ± 0.35			0.7 ± 0.15	
		Male	Female	Female			
Luteinizing hormone	ng/ml	10–40	20–40 (basal)	1500–2000 (proestrus)			
Follicle stimulating hormone	ng/ml		80–120 (basal)	250–300 (proestrus/estrus)			
Prolactin	ng/ml	<1	10–20				
Growth hormone	ng/ml		1–90				
Thyroid stimulating hormone	ng/ml		300				
Thyroxine	µg/dl	7.4 ± 0.5 (BALB/c)					
Corticosterone	µg/dl	9 (start of dark period) 5 (start of light period)	40 (middle of dark period)				
Epinephrine	pg/dl	0–200					
Norepinephrine	pg/dl	30–300					
Progesterone	ng/ml		5 (early proestrus)	35 (late proestrus, estrus)			
Estradiol	pg/ml		1–5 (basal)				
Testosterone	ng/ml	1.5–2.0					
Urine							
Volume	ml/16 hr	1.6 ± 0.9	1.7 ± 1.1				
Specific gravity		1.0341 ± 0.005					
pH		5.011					
Osmolality	Osm/kg	1.06–2.63					
Creatinine	mg/100g/24 hr	2.6 ± 0.91					
Glucose	mg/24 hr	0.53 ± 0.19					
Protein	mg/24 hr	0.7 ± 0.33	3.21 ± 1.05 (B6C3F1)				
Albumin	mg/ml	11.9 ± 0.2					

[a] Summarized from Loeb and Quimby (1999).

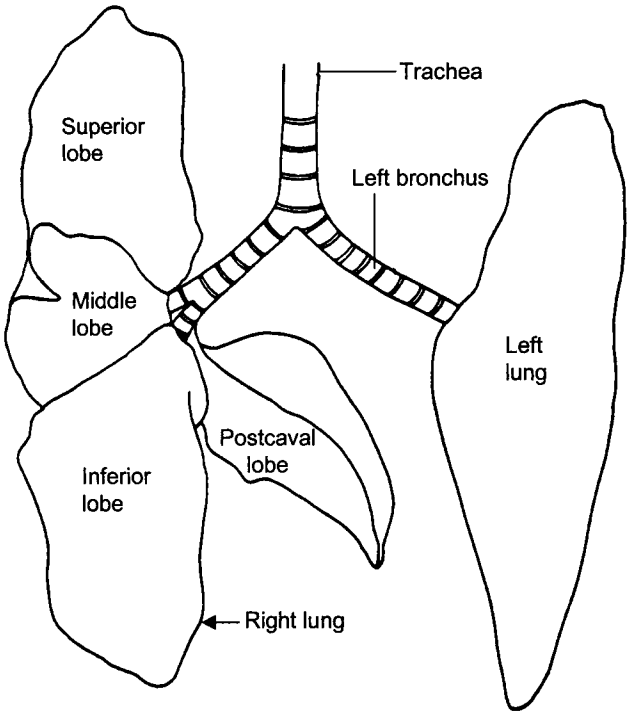

Fig. 3. Lobes of the lung. (From Cook, 1983.)

bronchi, all of which have cartilagenous support; and the posterior portion of the respiratory tract consists of the lungs. The left lung is a single lobe. The right lung is divided into four lobes: superior, middle, inferior, and postcaval (Fig. 3).

A mouse at rest uses about 3.5 ml O_2/gm/hr, which is about 22 times more O_2/gm/hr than is used by an elephant. To accommodate for this high metabolic rate, the mouse has a high alveolar P_{O_2}; a rapid respiratory rate; a short air passage; a moderately high erythrocyte (RBC) concentration; high RBC hemoglobin and carbonic anhydrase concentrations; a high blood O_2 capacity; a slight shift in the O_2-dissociation curve, enabling O_2 to be unloaded in the tissue capillaries at a high P_{O_2}; a more pronounced Bohr effect, i.e., the hemoglobin affinity for O_2 with changes in pH is more pronounced; a high capillary density; and a high blood sugar concentration.

3. Urinary System

The kidneys, ureters, urinary bladder, and urethra form the urinary system (Fig. 4). The paired kidneys lie against the dorsal body wall of the abdomen on either side of the midline. The right kidney is normally located anterior to the left kidney. Kidneys from males of many inbred strains are consistently heavier than kidneys from females. The glomeruli of mice are small, about 74 μm in diameter, or about half the size of glomeruli in

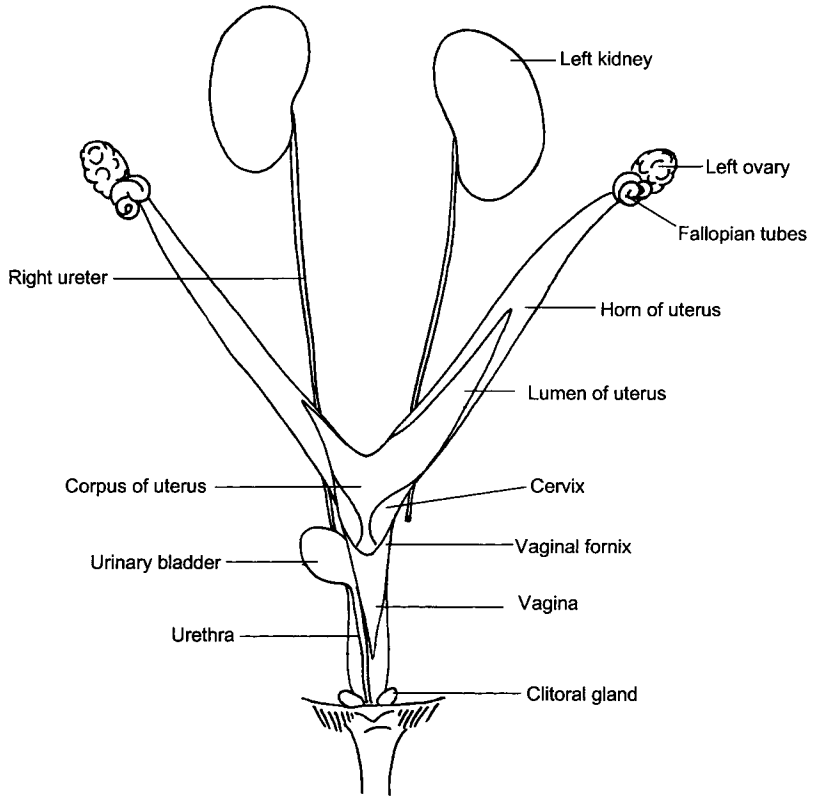

Fig. 4. Female urogenital tract. (From Cook, 1983.)

rats. There are, however, 4.8 times as many glomeruli in the mouse, and the filtering surface per gram of tissue is twice that of the rat.

Mice excrete only a drop or two of urine at a time, and it is highly concentrated (Table VIII). The high concentration is made possible by long loops of Henle and by the organization of giant vascular bundles (*vasa recta*) associated with the loops of Henle in the medulla. The mouse can concentrate urine to 4300 mOsm/liter, whereas the maximum permissible concentration is 1160 mOsm/liter for a human.

Mice normally excrete large amounts of protein in the urine. Taurine is always present in mouse urine, whereas tryptophan is always absent. Creatinine is also excreted in mouse urine, a trait in which mice differ from other mammals. The creatinine–creatine ratio for fasting mice is about 1:1.4. Mice excrete much more allantoin than uric acid.

4. Gastrointestinal Tract

The submaxillary salivary gland, a mixed gland in most animals, secretes only one type of saliva (seromucoid) in the mouse. The tubular portion of the gastrointestinal tract consists of esophagus, stomach, small intestine, cecum, and colon. The esophagus of the mouse is lined by a thick cornified squamous epithelium, making gavage a relatively simple procedure. The proximal portion of the stomach is also keratinized, whereas the distal part of the stomach is glandular. Gastric secretion continues whether or not food is present.

The gastrointestinal flora consists of more than 100 species of bacteria that begin to colonize the alimentary canal selectively shortly after birth. The ceca of normal mice contain up to 10^{11} bacteria/gm of feces. The bacteria throughout the gastrointestinal tract form a complex ecosystem that provides beneficial effects, such as an increase in resistance to certain intestinal pathogens, production of essential vitamins, and homeostasis of important physiological functions (Fig. 5).

Gnotobiotic animals colonized with known microbiota have been used to great advantage as models for biomedical research (Falk *et al.*, 1998; Wostman, 1996). For certain studies, it is desirable to colonize germfree mice with a defined microbiota. In the mid-1960s, Schaedler was the first to colonize germfree mice with selected bacteria isolated from normal mice (Schaedler and Orcutt, 1983). He subsequently supplied animal breeders with this group of microorganisms. These defined bacteria included aerobic bacteria and some less oxygen-sensitive anaerobic organisms. The so-called extremely oxygen-sensitive (EOS) fusiform bacteria, which make up the majority of the normal microbiota of rodents, were not included, because of technical difficulties in isolation and cultivation. Of the defined microbiotas later used for gnotobiotic studies, the one known as the "Schaedler flora" was the most popular. In 1978, the National Cancer Institute (NCI) decided to revise the Schaedler flora, or "cocktail" consisting of eight bacteria, in order to standardize the microbiota used to colonize germfree rodents. The new defined microbiota, now known as the "altered Schaedler flora" (ASF), consisted of four members of the original Schaedler flora (two lactobacilli, *Bacteroides distasonis,* and the EOS fusiform bacterium), a spiral-shaped bacterium, and three new fusiform EOS bacteria.

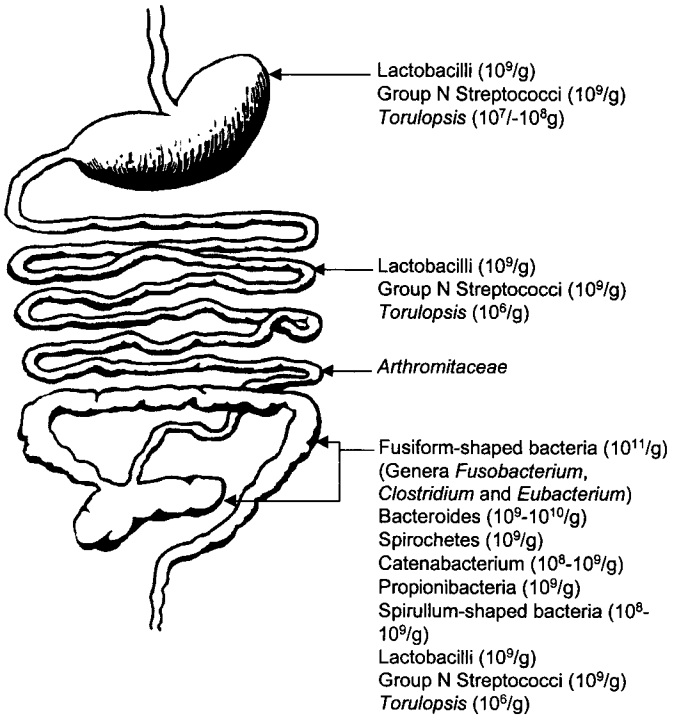

Fig. 5. Location of bacteria composing the autochthonous microflora in the gastrointestinal tract. (From Schaedler and Orcutt, 1983.)

It is difficult to monitor a gnotobiotic mouse colony with a defined microbiota. It is necessary to demonstrate that microorganisms of the specified microbiota are present and that adventitious microorganisms are absent. In the past, monitoring relied on bacterial morphology, limited evaluation of biochemical traits, and growth characteristics. Recently, the eight ASF strains were identified taxonomically by 16S rRNA sequence analysis (Dewhirst *et al.*, 1999). Three strains were previously identified as *Lactobacillus acidophilus* (strain ASF 360), *L. salivarius* (strain ASF 361), and *Bacteroides distasonis* (strain ASF 519), based on phenotypic criteria. 16S rRNA analysis indicated that each of the strains differed from its presumptive identity. The 16S rRNA sequence of strain ASF 361 is essentially identical to the 16S rRNA sequences of the type strains of *L. murinis* and *L. animalis* (both isolated from mice), and all of these strains probably belong to a single species. Strain ASF 360 is a novel lactobacillus that clusters with *L. acidophilus* and *L. lactis.* Strain ASF 519 falls into an unnamed genus containing [*Bacteroides*] *distasonis,* [*Bacteroides*] *merdae,* [*Bacteroides*] *forsythus,* and CDC group DF-3. This unnamed genus is in the *Cytophaga–Flavobacterium–Bacteroides* phylum and is most

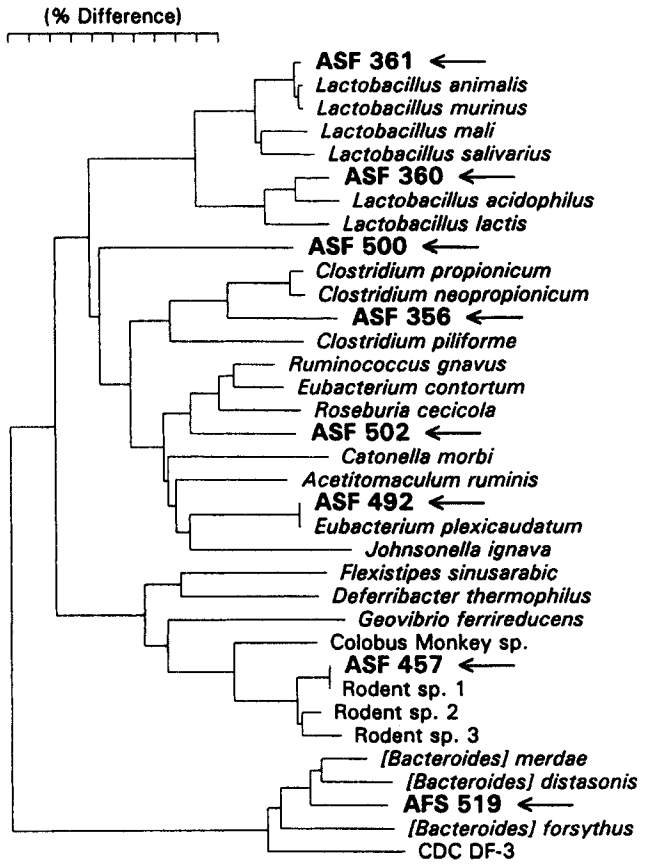

Fig. 6. Phylogenetic relationships of ASF strains. (Dewhirst *et al.*, 1999)

closely related to the genus *Porphyromonas*. The spiral-shaped strain, strain ASF 457, is in the *Flexistipes* phylum and exhibits sequence identity with rodent isolates of Robertson. The remaining four ASF strains, which are EOS fusiform bacteria, group phylogenetically with the low–G+C content gram-positive bacteria (*Firmicutes, Bacillus–Clostridium* group) (Fig. 6). The 16S rRNA sequence information determined by Dewhirst *et al.* (1999) should allow rapid identification of ASF strains and should permit detailed analysis of the interactions of ASF organisms during development of intestinal disease in mice that are coinfected with a variety of pathogenic microorganisms.

5. Lymphoreticular System

The lymphatic system consists of lymph vessels, thymus, lymph nodes, spleen, solitary peripheral nodes (Fig. 7), and intestinal Peyer's patches. Mouse lymph nodes are numerous but typically are small, reaching only a few millimeters. The typical lymph node is bean-shaped and consists of a cortex and a medulla. The cortex is divided into B lymphocyte domains, called primary follicles, and T lymphocyte domains, known as the diffuse cortex. The mouse does not have palatine or pharyngeal tonsils. The spleen lies adjacent to the greater curvature of the stomach. Different strains of mice have varying degrees of accessory splenic tissue. Age, strain, sex, and health status can affect the size, shape, and appearance of the spleen. Male spleens, for example, may be 50% larger than those of females. Most lymphocytes enter and leave the spleen in the bloodstream. The so-called white pulp of the spleen is organized along the central arteriole and is subdivided into T and B cell zones. The periarteriolar sheath is composed mainly of $CD4^+$ and $CD8^+$ T cells, and lymph follicles, which often contain germinal centers, are located at the periphery. The red pulp consists of sinusoids and hemoreticular tissue. Cellular and humoral components of immunity are distributed to the bloodstream and tissues by efferent lymphatic vessels and lymphatic ducts, which empty into the venous system.

The thymus is a bilobed lymphoid organ lying in the anterior mediastinum. It reaches maximum size around the time of sexual maturity and involutes between 35 and 80 days of age. The thymus plays a major role in maturation and differentiation of T lymphocytes. This function is not complete in newborn mice. Thymectomy is routinely performed in immunological research for experimental manipulation of the immune system. Thymectomy of newborn mice causes a decrease in circulating lymphocytes and marked impairment of certain immune responses, particularly cellular immune responses. Thymectomy in adult mice produces no immediate effect, but several months later mice may develop a progressive decline of circulating lymphocytes and impaired cellular immune responses. The mutant athymic nude mouse is a powerful experimental tool in the study of the thymus in immune regulation (Fogh, 1982).

The mucosa-associated lymph tissue (MALT) contains more lymphoid cells and produces greater amounts of immunoglobulin than both the spleen and the lymph nodes. The term *MALT* designates all peripheral lymphoid tissues connecting to cavities communicating with the external milieu. They include the Peyer's patches, the cecal lymphoid tissue, and the lymphoid tissue in upper and lower respiratory tract, as well as the genitourinary system. Lymphatics drain these lymphoid-rich areas, thus providing a direct link with lymph nodes and the bloodstream.

6. Blood and Reticuloendothelial System

Bone marrow and splenic red pulp produce erythrocytic, granulocytic, and megakaryocytic precursors over the life of the mouse. Bone marrow is located in the protected matrix of cancellous bone and is sustained by reticular tissue rich in blood vessels and adipose cells (Pastoret *et al.*, 1998). Normal hematologic values are listed in Table VII.

Bone marrow–derived mononuclear phagocytes remove particulate antigens and act as antigen-presenting cells for lymphocytes. Tissue macrophages, which often function in a similar way, are found in many tissues, including peripheral lymphoid tissues, lung, liver, intestine, and skin.

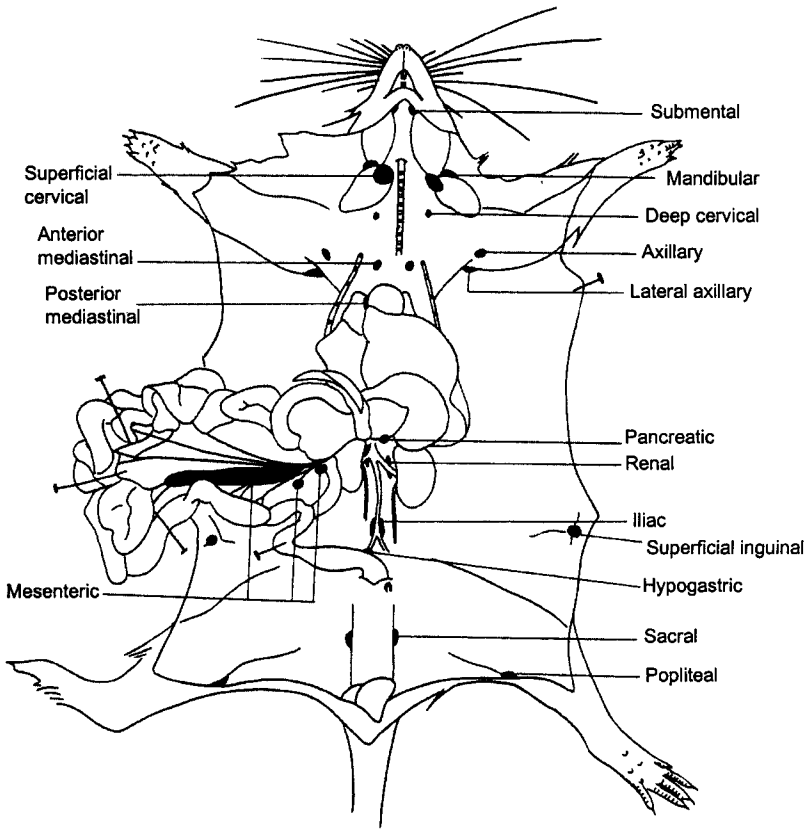

Fig. 7. Lymph nodes. (Modified from Cook, 1983.)

7. Cardiovascular System

The heart consists of four chambers, the thin-walled atria and the thick-walled ventricles (Fig. 8). Mice conditioned to a recording apparatus have mean systolic blood pressures ranging from 84 to 105 mm Hg. An increase in body temperature does not lead to an increase in blood pressure. Heart rate, cardiac output, and the width of cardiac myofibers are related to the size of the animal. Heart rates from 310 to 840/min have been recorded for mice, and there are wide variations in rates and blood pressure among strains.

8. Musculoskeletal System

The skeleton is composed of two parts: the axial skeleton, which consists of the skull, vertebrae, ribs, and sternum, and the appendicular skeleton, which consists of the pectoral and pelvic girdles and the paired limbs. The normal vertebral formula for the mouse is C7T13L6S4C28, with some variations among strains, especially in the thoracic and lumbar regions.

Normal mouse dentition consists of an incisor and three molars in each quadrant. These develop and erupt in sequence from front to rear. The third molar is the smallest tooth in both jaws; the upper and lower third molar may be missing in wild mice and in some inbred strains. The incisors grow continuously and are worn down during mastication.

9. Nervous System

The mouse brain has a typical mammalian structure. A detailed study of the neuroanatomy of the C57BL/6J mouse was made by Sidman *et al.* (1971).

10. Genital System

Female reproductive organs consist of paired ovaries and oviducts, uterus, cervix, vagina, clitoris, and paired clitoral glands (Fig. 4). The clitoral glands are homologous to the male preputial glands and secrete a sebaceous substance through ducts entering the lateral wall of the clitoral fossa. The female mouse normally has five pairs of mammary glands, three in the cervicothoracic region and two in the inguinoabdominal region. Detailed techniques for manipulating gametes and embryos have been developed (Daniel, 1978). The male reproductive organs consist of paired testes, urethra, penis, and associated ducts and glands (Fig. 9).

B. Reproduction

The following section summarizes normal reproduction in the mouse. The reader is referred to more comprehensive articles for additional information (Austin and Short, 1982; Rugh,

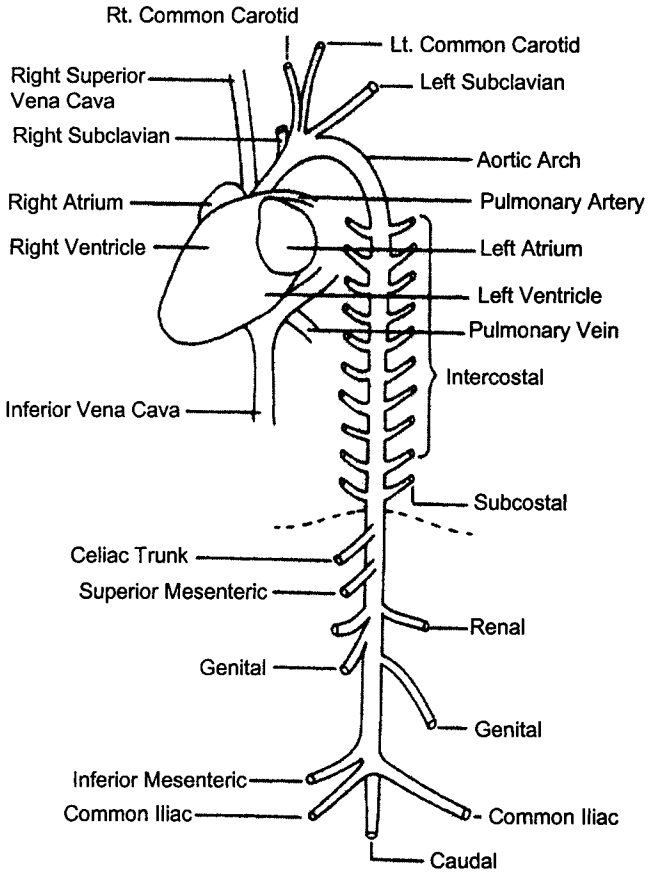

Fig. 8. Heart and major vessels. (Modified from Cook, 1983.)

1990; Whittingham and Wood, 1983). External influences, such as noise, diet, light, and population density, play an important role in reproduction and directly or indirectly influence the hypothalamic–pituitary axis for hormonal control of ovarian and testicular function. Genotype also dramatically affects the reproductive performance of the mouse.

1. Sexual Maturation

Follicle-stimulating hormone promotes gametogenesis in both sexes. Luteinizing hormones promote the secretion of estrogen and progesterone in the female and androgen in the male. Prolactin promotes lactation and development of the ovary during pregnancy. These gonadal hormones also ensure proper maintenance of the reproductive tract and modulate behavior to promote successful mating. The hypophysis is usually responsive to hormonal influence by day 6 in the male and day 12 in the female. Ovarian follicle development begins at 3 weeks of age and matures by 30 days. Rising levels of gonadotropins evoke signs of sexual maturity at about the same age. In the female, estrogen-dependent changes such as cornification of vaginal epithelium at the vaginal opening can occur as early as 24–28 days. Puberty is slightly later in the male (up to 2 weeks). Sexual maturation varies among strains and stocks of mice and is subject to seasonal and environmental influences. Mating behavior and the ability to conceive and carry fetuses to parturition are under complex hormonal control mediated by the anterior pituitary.

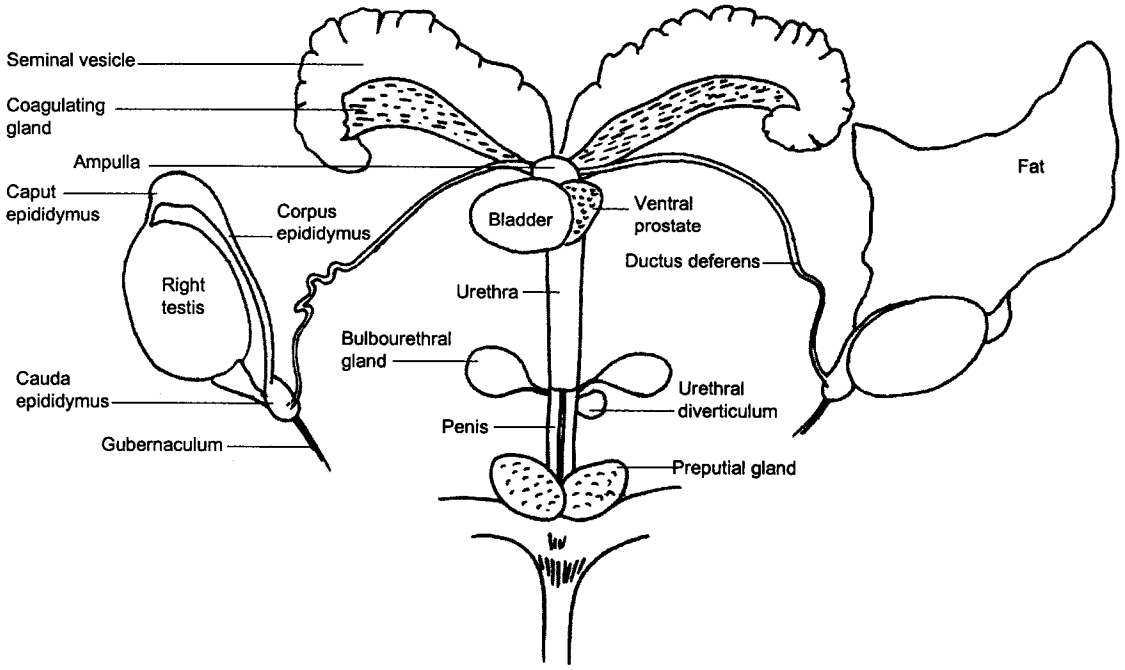

Fig. 9. Male reproductive tract. (From Cook, 1983.)

Table IX

Changes in the Reproductive Organs of the Mouse during the Estrous Cycle[a]

Stage	Smear[b]	Uterus	Ovary and oviduct
Proestrus	Epithelial cells to epithelial–cornified cells or epithelial–cornified cells; leukocytes to epithelial cells	Hyperemia and distension increases. Active mitoses in epithelium, few leukocytes	Follicles enlarged and distended with considerable liquor folliculi. Few mitoses in germinal epithelium and in follicular cells
Estrus	Epithelial–cornified cells to cornified + cells	Distension and activity are maximal during estrus and then decrease. No leukocytes	Ovulation occurs, followed by distension of upper end of oviduct. Active mitoses in germinal epithelium and in follicular cells
Metestrus	Cornified + + cells, epithelial cells, leukocytes + +	Distension decreased. Leukocytes in epithelium. Walls collapsed. Epithelium degenerates. Mitoses rare	Follicles undergoing atresia. Growing corpora lutea. Eggs in oviduct. Few mitoses in germinal epithelium and in follicular cells
Diestrus	Epithelial cells, leukocytes, more or less mucus	Pale in appearance, walls collapsed. Epithelium healthy but contains many leukocytes. Some secretion by uterine glands	Follicles begin rapid growth toward end of period

[a] Adapted from Bronson *et al.* (1996).

[b] + indicates many cells; + + indicates very many cells; – indicates transition from epithelial to cornified. The descriptions for smears are typical; there is considerable variation.

2. Estrous Cycle

The mouse is polyestrous and cycles every 4–5 days. In the first two phases (proestrus and estrus), active epithelial growth in the genital tract culminates in ovulation. Degenerative epithelial changes occur during the third phase, followed by diestrus, a period of quiescence or slow cell growth. The cycle can be followed by changes in the vaginal epithelium that are often used to determine optimum receptivity of the female for mating and fertilization (Table IX). Patency of the vaginal orifice and swelling of the vulva are useful signs of proestrus and estrus. Irregularities of the estrous cycle occur during aging. Seasonal and dietary factors, such as estrogenic substances found in a variety of feeds, and genetic backgrounds also influence estrous cycles.

Estrus is routinely observed in mice at about 14–24 hr after parturition (postpartum estrus). However, cornification of the vagina is not complete, and fertile matings are not as frequent compared with normal estrus. Mice are spontaneous ovulators. Ovulation does not accompany every estrus, and estrus may not coincide with every ovulation, because estrus is dependent on gonadal hormones, whereas ovulation is responsive to gonadotropin. The cyclicity of estrus and ovulation is controlled by the diurnal rhythm of the photoperiod. Mating, estrus, and ovulation most often occur during the dark phase of the photoperiod. Reversing the timing of the light–dark cycle reverses the time of estrus, ovulation, and mating.

Pheromones (Table X) and social environment also effect the estrous cycle. For example, estrus is suppressed in mice housed in large groups because of pseudopregnancy or diestrus ("Whitten effect"). These effects can be counteracted by olfactory stimuli evoked by chemical signals (pheromones) from male mice. By contrast, pheromones from a strange male mouse, particularly of a different strain, may prevent implantation or pseudopregnancy in recently bred females ("Bruce effect"). Estrus can be synchronized by group-housing females prior to pairing with males. Group housing suppresses estrus, but exposure to male pheromones restarts the cycle and leads to estrus in most females 3 days after pairing. The next estrus will occur in about 11 days.

Table X

Factors Leading to Pheromone Release in Mature Mice[a]

Initiator	Effect
Stressed mice	Dispersion of other mice
Females	Stimulate the approach, and sexual and aggressive behavior of males
"Foreign" females	Aggressive behavior by other females
Lactating females	Attract preweaning young
Males	Attract females
"Foreign" males	Aggression by other males
Males coexisting in a territory	"Foreign" males avoid the territory and inhibit aggressive tendencies of familiar males

[a] Modified from Shorey (1976).

3. Mating

Mating is normally detected by formation of a vaginal plug (a mixture of the secretions of the vesicular and coagulating glands of the male) whose prevalence is highly strain dependent. The plug usually fills the vagina from cervix to vulva (Fig. 10). Plug detection is often coupled with vaginal cytology to evaluate fertility and conception.

When the cervix and vagina are stimulated physically during estrus, prolactin is released from the anterior pituitary to enable the corpus luteum to secrete progesterone. Secretion continues for about 13 days. If fertilization has occurred, the placenta takes over progesterone production. If fertilization does not occur, a pseudopregnant period ensues, during which estrus and ovulation do not occur. Fertilization usually takes place in the ampulla or the upper portion of the oviduct. Ova can be fertilized to produce normal embryos for 10–12 hr after ovulation.

4. Gestation

Gestation is usually 19–21 days. Because of postpartum estrus, lactation and gestation can occur simultaneously. Lactation can delay gestation because of delayed implantation. This may cause prolongation of gestation for up to 12–13 days in certain inbred strains.

The effective reproductive life of some inbred strains approaches 2 years where optimum environmental conditions are maintained, but litter size usually decreases as the female ages. Therefore, females are usually retired by 1 year of age. Average litter size is strain dependent and commonly ranges from 1 to 12 pups.

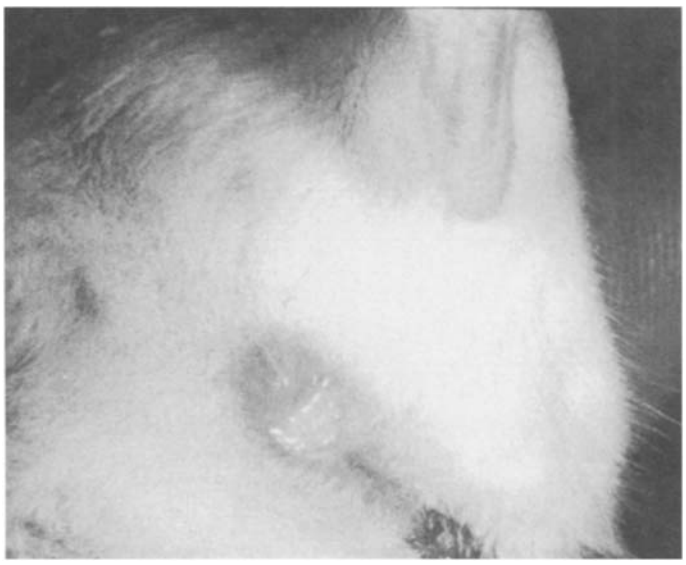

Fig. 10. Vaginal plug. (Courtesy of Laboratory Animal Medicine and Science Autotutorial Series.)

5. Postnatal Development and Weaning

Maternal care can account for about 70% of the variation in body weight of neonatal mice. Nursing females usually lactate for 3 weeks. Milk production increases up to 12 days postpartum and then declines until weaning at 21 days. Interestingly, oxytocin is required for nursing but is not essential for parturition or reproductive behavior (Nishimori *et al.*, 1996).

Some transmission of humoral immunity from dam to progeny occurs *in utero,* but the majority of antibody is transferred through colostrum. Transmission of passive immunity by colostral antibodies has been demonstrated to a wide variety of antigens, including viruses, bacteria, and parasites. Antibodies continue to be secreted in the milk throughout lactation. Decay of maternally acquired immunity occurs within several months after weaning. Loss of maternal immunity increases susceptibility to infection and warrants continued care of weaned mice under barrier conditions.

C. Behavior

Mice are social mammals in which pheromones play an important role in communication (Table X). Pheromones have been divided into two broad categories: primer pheromones and releaser pheromones (Wilson, 1970). Primer pheromones are probably detected by the vomeronasal organs, which relay messages to the central nervous system (CNS), resulting in modulated behavior. Releaser pheromones trigger a prompt CNS-mediated behavioral response in a recipient. Pheromones most frequently affect developmental and reproductive processes. Primer pheromones produced by males also regulate the reproductive physiology of female mice (Keverne, 1998). More than one response can be elicited by the same pheromone. For example, small, structurally diverse ligands, which bind to the major urinary protein of male mice, demonstrate puberty-accelerated pheromone activity in recipient females. Four of these ligands have been implicated in estrus sychronization (Whitten effect). However, the same chemosensory substances now appear to be responsible for both sexual maturation and estrus cycling in female mice (Novotny *et al.*, 1999). Pheromone communication must be accounted for in the management of mouse colonies, particularly when subtle behavioral traits or reproductive performance are critical (Ma *et al.*, 1998).

Behavior in mice also is determined by genotype and environment. Male BALB/c mice, for example, are prone to fights and have a high prevalence of bite wounds around the head, back, shoulders, perineum, and tail. Aggressive behavior can sometimes be diminished by caging together only males from one litter or males paired prior to weaning. Hair nibbling and whisker chewing are examples of social dominance (Fig. 11). These traits may be an exaggeration of inherited grooming

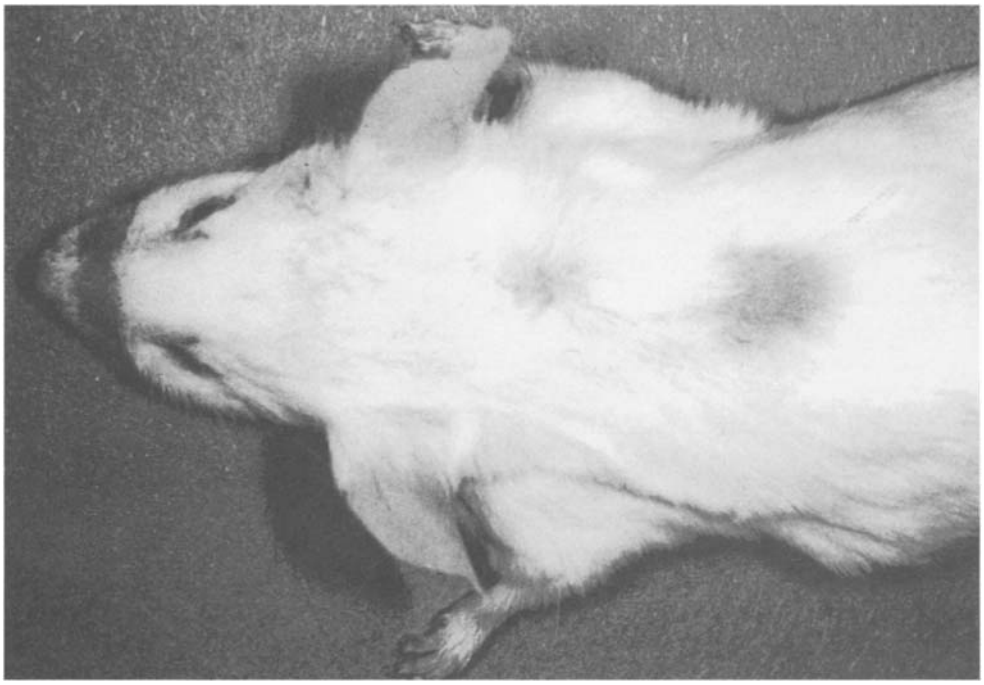

Fig. 11. Barbering. (Courtesy of Dr. J. G. Fox.)

behavior. Dominant mice usually retain their whiskers—hence the name *barber mouse.* Hair loss from barbering must be distinguished from hair loss due to ectoparasitism, microbial dermatitides, and abrasions from improperly designed cage covers. Maintenance behavior, such as eating and drinking, is cyclic and occurs mostly during the evening or at night. Nest building is another important social behavior and can be observed by placing a nestlet cotton square or other nesting material in the mouse's cage.

Mice, and particularly genetically engineered mice, are being increasingly used in behavioral research (Crawley, 1999). About 100 different genes have been studied thus far in the mouse central nervous system. They have been identified and their phenotype ascertained in transgenic and knockout mice (Bedell *et al.,* 1997; Campbell and Gold, 1996; Nelson and Young, 1998).

Initial behavioral evaluations include general health and neurological reflexes assessment. Sensory abilities and motor functions are extensively quantitated. Specific tests include observations of home cage behaviors, body weight, body temperature, appearance of the fur and whiskers, righting reflex, acoustic startle, eye blink, pupil constriction, vibrissae reflex, pinna reflex, Digiscan open field locomotion, rotarod motor coordination, hanging wire, footprint pathway, visual cliff, auditory threshold, pain threshold, and olfactory acuity. Hypothesis testing then focuses on at least three well-validated tasks within each relevant behavioral domain. Specific tests for mice are being utilized for the domains of learning and memory, feeding nociception, and behavior that are relevant to discrete symptoms of human anxiety, depression, schizophrenia, and drug addiction (Crawley, 1999). Substantial effort also is being directed to optimize behavioral phenotyping in mice.

D. Immunology

The mouse is the primary mammalian model for immunology research because of the extensive literature available on this species, the availability of immunological reagents, the soon-to-be-published complete mouse genome, and numerous genetically defined mouse strains, as well as the ever increasing number of genetically manipulated mice with defined genetic alterations. Unless otherwise indicated, information in this section is from Pastoret *et al.* (1998).

1. Immunoglobulins

The mouse has five classes of immunoglobulins, which are determined by the heavy constant region and are classified by isotype, IgM, IgD, IgG, and IgA. The IgG class is further divided into IgG_1, IgG_{2a}, IgG_{2b}, and IgG_3. The humoral immune response varies according to the type of immunogen used to evoke the response and the immunoglobulin isotypes that express the effects or functions. For example, IgM is secreted after initial exposure to an antigen, followed by IgG, the most abundant antibody in serum. In viral infections or intracellular bacterial infections, IgG_{2a} is dominant, whereas in parasitic infection IgG_1 is dominant. IgG_{2b} and IgG_3 are usually induced by T cell–independent antigens, such as carbohydrates. IgG_3 also

is important in responding to bacterial antigens, e.g., by playing a role in phagocytosis. IgE is linked to allergy, and IgA plays a pivotal role in mucosal immunity (Kramer and Cebra, 1995). Finally, IgD plasma cells are rarely found, and their exact function remains an enigma.

2. Cellular Immunology

Murine lymphoid cells can be phenotyped by monoclonal antibodies against surface markers and are referred to as cluster of differentiation, or CD, antigens. The nomenclature of the cells that express these antigens and other proteins that recognize them and their function have been extensively determined in the mouse, and these markers are routinely used to characterize pathological processes in mouse models. Mouse T lymphocytes can be differentiated into two primary phenotypes, based on their expression of CD molecules. $CD4^+$ T cells (helper T cells) are MHC class II restricted and promote B lymphocyte responses essential for humoral immunity. $CD8^+$ T cells are MHC class I restricted and serve as cytotoxic cells for cell-mediated immunity (e.g., against cells containing infectious agents) or act to suppress immune responsiveness.

3. Cytokines

Cytokines are a set of signaling molecules involved in cells communicating with one another in a complex biological system. Cytokine-mediated signaling primarily occurs after initiation and effector events of the host's immune response and in the development of hematopoietic cells. Because of their importance in modulating tissue responses to antigenic stimuli, a number of mouse cytokines have been described and are routinely used in research (Table XI).

4. Models of Immune Dysregulation

A number of spontaneous mouse models of immune deficiency have been used extensively in research (Table XII). Their use—plus the expanding number of immune dysregulated knockout, transgenic, and dominant negative mutants—has advanced understanding of human immune deficiency diseases as well as basic understanding of the immune system. Investigators using genetically engineered mice are constantly reminded, however, that phenotypic analysis of these animals must be done cautiously because the immune system may be profoundly affected and in ways that are not always anticipated. This may make it difficult to determine whether a given gene product is directly involved or may be secondary to a more global dysregulation of the immune system. As with other biological systems, compensation mechanisms also may mask the phenotype. Use of dominant negative mutations has reduced this possibility somewhat, because mice express transgenes encoding for abnormal, catalytically inactive proteins, which in turn block a given pathway despite the existence of redundant pathways.

III. DISEASES

Contemporary knowledge about diseases of laboratory mice has developed primarily from examining the effects of disease on traditional strains and stocks. The widespread use of genetically engineered mice is likely to modify current concepts because of novel or unpredictable interactions among genetic alterations, the genetic backgrounds on which they are expressed, and exogenous factors, such as infectious agents. Because the number of combinations is extraordinarily high, clinical and laboratory diagnosticians should be alert to the potential for altered disease expression in genetically engineered mice and not be misled by unexpected signs, lesions, and epizootiology.

A. Infectious Diseases

Microbiological Surveillance

Many of the agents and conditions discussed under Section III,A, may interfere with mouse-based research. Housing and husbandry in microbiologically sheltered environments are designed to reduce the risks of disruptive infection, especially among immunologically dysfunctional mice, but must be accompanied by effective microbiological surveillance. Several principles are worthy of emphasis at the outset. (1) Surveillance should encompass resident mice *and* mouse products (serum, cell lines, transplantable tumors) procured from external sources. Many commercial vendors currently provide sound, contemporary microbiological data on animals that they sell. Further, it is impractical, logistically and financially, to test every batch of commercially procured animals. However, thorough quarantine and testing of mice and mouse products from noncommercial sources should be mandatory. (2) Testing should be consonant with institutional needs. Therefore consideration must be given to the list of agents for which testing will be done, the minimum prevalence level for a given infection that testing is designed to detect, the frequency of testing, the number and location of animals to be tested per unit of time, sample collection strategies, and costs. Additionally, it is important to consider the impact of modern housing systems on detection strategies. Microbarrier cages, while protecting mice against infection, may also limit detection of low-level infection by impeding cage-to-cage transmission. Therefore detection should include preemptive exposure of sentinel mice to multiple bedding samples or the complementary use of molecular diagnostics to sample animals, soiled bedding, or spent air from ventilated cage racks. (3) The tests selected should provide a high degree

Table XI

Major Sources, Cellular Targets, and *in Vivo* Effects of Select Mouse Cytokines[a]

Cytokine	Cell source	Cell targets	Function
IFN-α, IFN-β	Macrophages, B and T cells, fibroblasts, epithelial cells	Many cell types	Antiviral, antiproliferative, stimulate NK activity and macrophage functions
IFN-γ	T cells, NK cells	Macrophages, lymphocytes, NK cells	Proinflammatory, promotes Th1 immune responses/ secretion of Th1-associated cytokines
IL-1α, IL-1β	Macrophages, endothelial cells, keratinocytes, lymphocytes, fibroblasts, osteoblasts	Many cell types	Proinflammatory, stimulates fibroblasts and bone catabolism, neuroendocrine effects (fever, sleep, anorexia, corticotropin release)
IL-2	Activated T cells	Macrophages, T and B cells, NK cells	T cell growth factor, stimulates NK activity
IL-3	T cells, mast cells	Mast cells, hematopoietic progenitors	Promotes proliferation and differentiation of mast cell and hematopoietic cell lineages (granulocytic, monocytic, megakaryocytic)
IL-4	T cells, basophils, mast cells, bone marrow stromal cells	B and T cells, mast cells, macrophages, hematopoietic progenitors	Proliferation and differentiation of B cells (Ig switching to IgG_1 and IgE) and Th2 cells (anti-inflammatory by inhibiting Th1 immune responses)
IL-5	T cells, mast cells	Eosinophils, B cells	Stimulates eosinophilia, growth and differentiation of B cells, Ig switching
IL-6	Fibroblasts, macrophages, endothelial cells, T cells	B and T cells, thymocytes, hepatocytes, neurons	Differentiation of myeloid cells, induction of acute phase proteins, tropic for neurons
IL-7	Thymic and bone marrow stromal cells	B and T cells	Growth factor for B and T cells
IL-8	Monocytes, neutrophils, fibroblasts, endothelial cells, keratinocytes, T cells	Neutrophils, basophils, T cells	Proinflammatory, activates neutrophils, enhances keratinocyte growth
IL-9	T cells	$CD4^+$ T cells, mast cells	Enhances hematopoiesis
IL-10	Macrophages, T and B cells, mast cells, keratinocytes	Macrophages, T and B cells	Anti-inflammatory Th2 immune responses, inhibits Th1 responses
IL-11	Stromal cells	Hematopoietic progenitor cells	Hematopoiesis
IL-12	T cells	T cells, macrophages	Proinflammatory; promotes NK and cytotoxic lymphocyte activity; induces IFN-γ, which in turn promotes Th1 immune responses
IL-13	T cells	B cells	Activation of Ig transcription, key mediator in asthma
IL-14	Endothelial cells, lymphocytes	B cells	B cell growth factor
IL-15	Fibroblasts, keratinocytes, endothelial cells, and macrophages	T and B cells, NK cells, monocytes, eosinophils, neutrophils	Enhances neutrophil chemokine production, cytoskeletal rearrangements, phagocytosis; delays apoptosis
IL-16	Epithelial cells, mast cells, $CD4^+$ and $CD8^+$ cells, eosinophils	$CD4^+$	$CD4^+$ T cell growth factor; proinflammatory; enhances lymphocyte chemotaxis, adhesion molecule and IL-2 receptor and *HLA-DR* expression
IL-17	Human memory T cells, mouse αβ TCR^+ $CD4^-$ $CD8^-$ thymocytes	Fibroblasts, keratinocytes, epithelial and endothelial cells	Secretion of IL-6, IL-8, PGE2, MCP-1 and G-CSF, induces *ICAM-1* expression, T cell proliferation
IL-18	Macrophages, keratinocytes, microglial cells	T cells; NK cells; myeloid, monocytic, erythroid, and megakaryocytic cell lineages	Proinflammatory, induces IFN-γ and other Th1 cytokines, promotes Th1 development and NK activity
GM-CSF	Macrophages, stromal cells, fibroblasts, endothelial cells, lymphocytes	Hematopoietic stem cells, neutrophils, macrophages	Growth and differentiation of granulocytes, macrophages
TNF	Macrophages, T and B cells, NK cells	Many cell types	Proinflammatory, fever, neutrophil activation, bone resorption, anticoagulant, tumor necrosis
TGF-β	Platelets, macrophages, T and B cells, placenta, hepatocytes, thymocytes	Many cells types	Anti-inflammatory; promotes wound healing, angiogenesis; suppresses hematopoiesis, lymphopoiesis, Ig production, NK activity; promotes Ig switching to IgA

[a] IFN, interferon; IL, interleukin; GM-CSF granulocyte–macrophage colony stimulating factors; NK, natural killer; TNF, tumor necrosis factor; TGF, tumor growth factor.

Table XII

Common Mouse Models of Immunodeficiency

Model	Immunodeficiency	Phenotype	Major uses
Nude mouse	Defective transcription factor gene controlling thymic epithelial cell differentiation	Athymic and hairless (unrelated but linked gene defect) No T cell functions	Tumor and xenograft studies
SCID mouse	Defective DNA-dependent kinase that recombines gene segments coding for T (TcR) and B (Ig) cell receptors	Hypoplastic lymphoid tissues No Ig or T cell responses Sensitive to ionizing radiation because of defective DNA break repair	V (D)J recombination studies Tumor and xenograft transplantation Lymphocyte subset transfer studies Reconstitution of human hematopoietic system (Hu-PBL-SCID)
Rag-1 and Rag-2 mice	Defective recombinase enzymes (Rag-1 and/or Rag-2), preventing formation of functional B α (Ig) and T (TcR) cell receptors	Hypoplastic lymphoid tissues No Ig or T cell responses	V (D)J recombination studies Tumor and xenograft transplantation Lymphocyte subset transfer studies
XID mouse	Defect in Bruton's tyrosine kinase gene affecting signal transduction in B cells	Decreased B cell numbers, low IgM Impaired response to polysaccharide antigens	Model for human X-lined agammaglobulinemia
Moth-eaten mouse	Defective phosphatase, impairing signal transduction from cell receptors	Deficient humoral and cellular immunity Lack cytotoxic T and NK cells Moth-eaten pelage secondary to folliculitis Autoimmune syndromes Hypergammaglobulinemia	Apoptosis studies Autoimmune syndromes
Beige mouse	Mutation on chromosome 13 affects pigment granules (coat, retina) and lysosomal granules of type II pneumocytes, mast cells, and NK cells	Diluted coat color Lysosomal storage disease Impaired chemotaxis, bactericidal activity of neutrophils, decreased NK activity	Model for Chediak–Higashi syndrome Crossed onto nude or SCID backgrounds for multiple imune deficiencies
lpr and gld mice	Impaired apoptosis from Fas (lpr) or Fas ligand (gld) defect	Generalized lymphoproliferative disease (gld), autoimmunity, immunodeficiency	Apoptosis studies Autoimmune syndromes
Cytokine KO mice (IL-2, IL-10, IFN-γ, TNF-β, others)	Genetically engineered disruption (knockout) of cytokine gene	Anemia (IL-2), wasting (IL-2, IL-10), and inflammatory bowel disease (IL-2, IL-10) when housed conventionally	Physiological role of cytokines in immune response and inflammation
Receptor KO mice (TcR, Ig, cytokine, MHC, adhesion molecules, integrins)	Genetically engineered disruption (knockout) of receptor gene	Lack functional response to signal of interest, variable immune compromise Inflammatory bowel disease common in TcR KO	Physiological role of receptors in immune response and inflammation

of sensitivity and specificity. The traditional benchmark for testing is serology, but molecular diagnostics, microscopy (especially for parasites), and isolation of the agent (especially of bacteria) may be justified for detection or verification. (4) Interpretation of test results and resulting strategies to eliminate or contain infection should be based on a thorough knowledge of the agent under consideration, its potential effects on mice knowingly or potentially exposed, and the validity of the testing and surveillance methods.

Because effective surveillance strategies will vary with research needs and operating conditions, it is prudent to consult at least several literature sources about options for testing and monitoring for infectious agents and disease before launching or modifying a surveillance program (Barthold, 1998; de Souza and Smith, 1989; FELASA, 1994, 1996; Lindsey *et al.*, 1991a; Lussier, 1991; Nicklas *et al.*, 1993; Rehg and Toth, 1998; Small, 1984; Waggie *et al.*, 1994; Weisbroth *et al.*, 1998; White *et al.*, 1998). There are also recommendations regarding specific agents in following sections.

1. Viral Diseases

a. Mousepox (Fenner, 1948, 1982, 1990)

Etiology. Mousepox is caused by ectromelia virus, an orthopoxvirus that is closely related antigenically and physicochemically to vaccinia virus. Field strains of ectromelia virus have been isolated in many countries, but several, including Hampstead (low virulence), Moscow (high virulence), and NIH-79 (high virulence), have been used extensively for laboratory

study. Ectromelia virus grows well on the chorioallantoic membrane of embryonated chicken eggs and can also infect HeLa cells, mouse fibroblasts (L cells), and chick embryo fibroblasts. The BS-C-1 cell line is particularly sensitive to infection. Ectromelia virus produces an envelope hemagglutinin whose detection forms the basis for hemagglutination inhibition, a historically important serological test for mousepox.

Clinical signs. Mousepox usually takes one of three clinical courses: acute asymptomatic infection, acute lethal infection, or chronic infection with low mortality. The expression of clinical signs reflects an interplay among virus-related factors, including virulence and dose, and host-related factors, including age, genotype, immunological competence, and portal of entry. Acute lethal infection occurs in genetically susceptible strains (Briody, 1959). Outbreaks among susceptible mice are often volatile, with variable morbidity and high mortality. Clinical signs such as ruffled fur or prostration, may occur for only a few hours before death. This rapidly fatal form is associated with multisystemic necrosis. Mice that survive acute infection can develop chronic disease characterized by a skin rash whose severity depends on the extent of viremia secondary to infection of parenchymal organs. The rash can develop anywhere on the body and may be focal or generalized (Fig. 12). Conjunctivitis also may occur. The skin lesions usually recede within several weeks, but hairless scars can remain. Additionally, severe viral infection of the feet and tail during the rash syndrome can lead to necrosis and amputation.

Epizootiology. Mousepox is not a common disease. Outbreaks occur sporadically and can usually be traced to the importation of contaminated mice or mouse products. For example, contaminated mouse serum was responsible for recent outbreaks in the United States (Dick *et al.,* 1996; Lipman *et al.,* 2000). Natural exposure is thought to occur through skin abrasions, but oral inoculation can cause chronic inapparent infection of Peyer's patches accompanied by prolonged excretion of virus in feces. Mice with chronic skin disease can transmit infection by contact and also are a source of contaminated tissues. Intranasal inoculation can produce necrotizing rhinitis and pneumonia in addition to systemic disease. However, cage-to-cage transmission of infection is low and can be virtually nil if cage bonnets are employed (Bhatt and Jacoby, 1987b). Ectromelia virus is highly stable at room temperature, especially under dry conditions, leading to the potential for prolonged environmental contamination in infected colonies (Bhatt and Jacoby, 1987c). Aerogenic exposure is not a major factor in natural outbreaks, and arthropod-borne transmission does not appear to occur. However, intrauterine infection and fetal deaths have been reported. Therefore contamination of cesarean-derived progeny or embryo-derived cell cultures is possible.

The mouse is the only known host for ectromelia virus. Genotype can modulate the course and severity of infection. DBA/1, DBA/2, BALB/c, A, and C3H mice are among the inbred strains most susceptible to lethal infection, whereas C57BL/6 and AKR are resistant (Wallace, *et al.,* 1985; Bhatt and Jacoby, 1987a). Immunodeficient mice also should be considered highly susceptible. The mechanisms of genetic resistance are not fully understood but appear to reflect multiple genes, some of which appear to be expressed through lymphoreticular cells, including natural killer cells (Brownstein and Gras, 1995, Jacoby *et al.,* 1989).

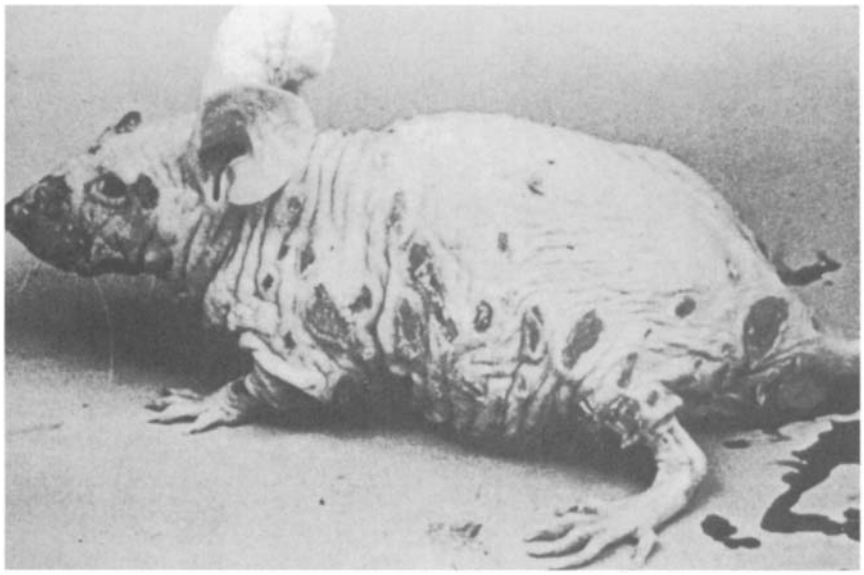

Fig. 12. A hairless mouse with mousepox. (From Fenner, 1982, and with permission of the Zentral Institut für Veruchstiere, Hannover, Federal Republic of Germany.)

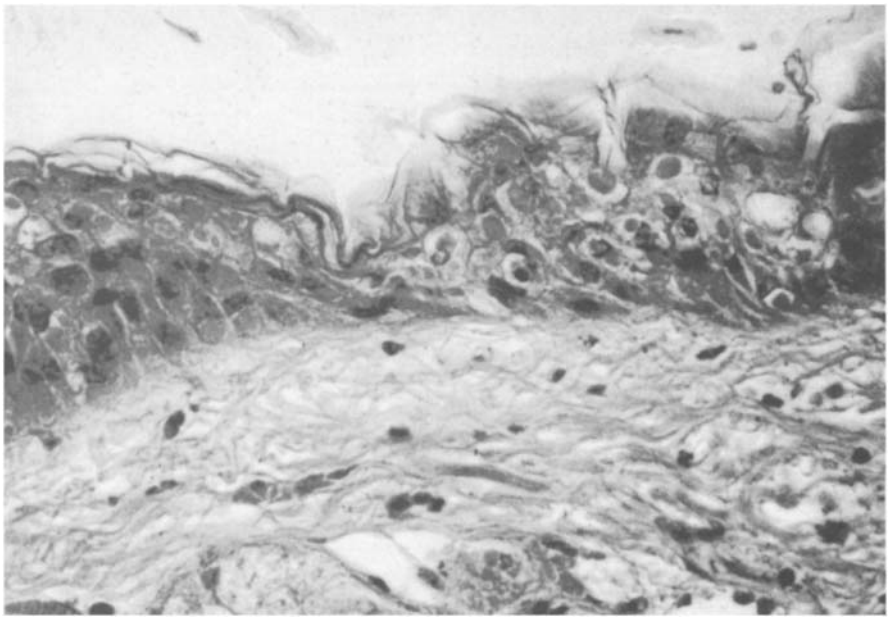

Fig. 13. Skin with intracytoplasmic type A inclusions of ectromelia virus.

Natural transmission is facilitated by intermediately resistant mice. They frequently survive long enough to develop skin lesions that can shed virus and serve as a major reservoir for spread of infection. The risks for transmission are further increased by persistence of infectious virus in excreta and exfoliated scabs. Although virus excretion typically lasts for about 3 weeks, virus has been found in scabs and/or feces for up to 16 weeks. Resistant mouse strains also are dangerous because they can shed virus during asymptomatic infections. However, infections in resistant mice tend to be short-lived. Ironically, highly susceptible mice are a relatively small hazard for dissemination of infection, if properly discarded, because they die before virus shedding becomes prominent. Thus, the juxtaposition of enzootically infected and highly susceptible mice can provoke explosive outbreaks. Infant and aged mice are usually more susceptible to lethal infection than young adult mice. Maternal immunity among enzootically infected breeding mice may perpetuate infection by protecting young mice from death, but not from infection. Such mice may subsequently transmit infection by contact exposure.

Pathology. Ectromelia virus multiplies in the cell cytoplasm and produces two types of inclusion bodies. The A type (Marchal body) is well demarcated and acidophilic in standard histological sections. It is found primarily in epithelial cells of skin or mucous membranes and can also be found in intestinal mucosa (Fig. 13). The B type of inclusion is basophilic and can be found in all ectromelia-infected cells. However, it is difficult to visualize unless cells are stained intensely with hematoxylin or, preferably, by immunohistochemistry for ectromelia virus antigens on formalin-fixed, paraffin-embedded tissue sections (Jacoby and Bhatt, 1987) (Fig. 14).

Following skin invasion, viral multiplication occurs in the draining lymph node and a primary viremia ensues (Fig. 14). Splenic and hepatic involvement begin within 3–4 days, whereupon larger quantities of virus are disseminated in blood to the skin. This sequence takes approximately 1 week and, unless mice die of acute hepatosplenic infection, ends with the development of a primary skin lesion at the original site of viral entry. The primary lesion is due to the development of antiviral cellular immunity.

Severe hepatocellular necrosis occurs in susceptible mice during acute stages of mousepox. White spots indicative of necrosis develop throughout the liver. In nonfatal cases, regeneration begins at the margins of necrotic areas, but inflammation is variable. Splenic necrosis in acute disease commonly precedes hepatic necrosis but is equally or more severe (Fig. 15). Necrosis and scarring of red and white pulp can produce a macroscopic "mosaic" pattern of white and red-brown (Fig. 16). Necrosis of thymus, lymph nodes, Peyer's patches, intestinal mucosa, and genital tract also have been observed during acute infection, whereas resistant or convalescent mice can develop lymphoid hyperplasia. Severe intestinal infection may be accompanied by hemorrhage.

The primary skin lesion, which occurs 6–10 days after exposure, is a localized swelling that enlarges from inflammatory edema. Necrosis of dermal epithelium provokes a surface scab and heals as a deep, hairless scar. Secondary skin lesions (rash) develop 2–3 days later as the result of viremia. They are often multiple and widespread and can be associated with

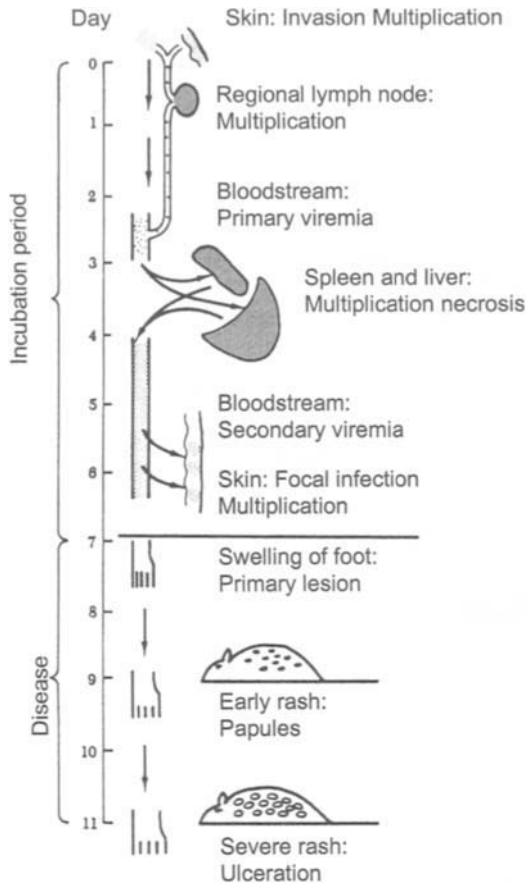

Fig. 14. Diagram illustrating the pathogenesis of mousepox. (From Fenner, 1948.)

conjunctivitis, with blepharitis, and, in severe cases, with buccal and lingual ulcers. The skin lesions also can ulcerate and scab before scarring.

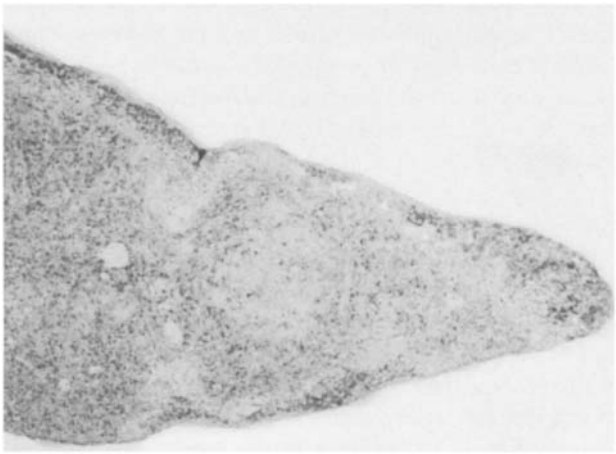

Fig. 15. Splenic necrosis in acute mousepox.

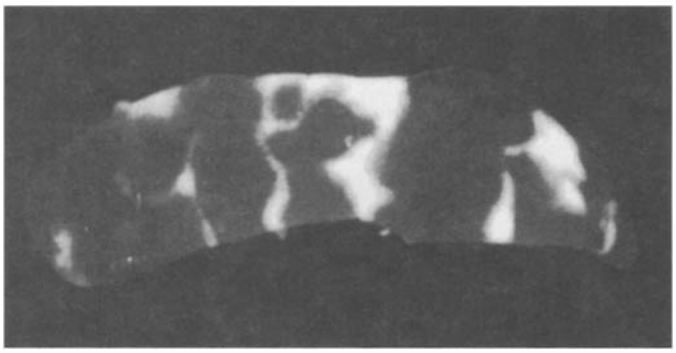

Fig. 16. "Mosaic spleen" from a mouse that survived acute mousepox.

Diagnosis. Mousepox can be diagnosed from clinical signs, lesions, serological tests, and demonstration of virus or viral antigen in tissues. Detection of characteristic intracytoplasmic eosinophilic inclusions aids detection of infection. Virus can be isolated from infected tissues by inoculation of cell cultures (BS-C-1) or embryonated eggs. Several serological tests are available to detect mousepox. Historically, the standard test was hemagglutination inhibition (HAI), using vaccinia antigen as a source of hemagglutinin. An enzyme-linked immunosorbent assay (ELISA) is more sensitive and specific and has replaced HAI for serological monitoring among nonvaccinated mice (Buller *et al.,* 1983). Ectromelia virus infection also can be detected by an immunofluorescence assay (IFA) and a PCR (polymerase chain reaction) assay (Neubauer *et al.,* 1997). Serological differentiation of mousepox from vaccinia infection in vaccinated mice is based on the lack of hemagglutinin in the vaccine strain of virus. Thus, serum from vaccinated mice may react by ELISA but should not react by HAI.

Differential diagnosis. Mousepox must be differentiated from other infectious diseases associated with high morbidity and high mortality. These include mouse hepatitis, Tyzzer's disease, and reovirus 3 infection. Each can be expressed by acute necrosis in parenchymal organs, but they can be differentiated by morphological, serological, and virological criteria. The skin lesions of chronic mousepox must be differentiated from other skin diseases caused by opportunistic or pathogenic bacteria, acariasis, and bite wounds.

Prevention and control. Mousepox is a dangerous disease because of its virulence for susceptible mice. Therefore, infected colonies should be quarantined immediately. Depopulation coupled with vaccination has been used as a primary means for control, but confirmation of infection should be obtained before exposed mice are destroyed. Tissues, supplies, instruments, or other items that have had potential contact with infected mice should be disinfected by heat or chemicals such as formalin, sodium hypochlorite, or chlorine dioxide. Materials should be autoclaved or, preferably, incinerated. Disinfected rooms should be challenged with susceptible sentinel animals that are ob-

served for clinical signs and tested for seroconversion after several weeks. Depopulation and disinfection must be done vigorously. Because modern housing and husbandry methods based on the use of microbarrier caging are effective for containing infection, testing and culling properly isolated mice is a potential alternative, especially for irreplaceable breeding mice, such as transgenic founders. Such mice can be quarantined along with cessation of breeding to permit resolution of infection (Bhatt and Jacoby, 1987b). Sequential testing with contact-exposed sentinels should be employed with this option. Additionally, maternal immunity from fully recovered dams can protect mice from infection, thereby enhancing opportunities to derive virus-free mice from previously infected dams.

Vaccination can control or prevent clinically apparent mousepox. The hemagglutinin-deficient strain of vaccinia virus (IHD-T) is used to scarify skin on the dorsum of the tail. "Takes" should occur in previously uninfected mice by 6–10 days, but not in infected mice (Bhatt and Jacoby, 1987d) (Fig. 17). Infected mice should be quarantined separately or eliminated. Vaccination may not prevent infection, although infection in vaccinated mice is often transient. Furthermore, vaccinia virus can be shed from scarification sites for at least several days. Therefore, other preventive measures, such as strict controls on the entry of mice or mouse products, combined with periodic serological monitoring, should not be relaxed until diagnostic testing has confirmed the elimination of vaccinia and ectromelia virus. Additionally, seroconversion evoked by vaccination must be taken into account in serological monitoring of vaccinated colonies. Finally, vaccinia virus is a human pathogen, so vaccination procedures should include personnel protective measures to prevent exposure.

Research complications. The primary threat from mousepox is mortality in susceptible mice. The loss of time, animals, and financial resources can be substantial.

b. Herpesvirus Infections (Osborn, 1982, 1986)

Mice are naturally susceptible to two herpesviruses: mouse cytomegalovirus and thymic necrosis virus. They are species-specific viruses and antigenically distinct from each other and from other rodent herpesviruses.

i. Mouse cytomegalovirus (MCMV) infection (Lussier, 1994)

Etiology. MCMV is a mouse-specific betaherpesvirus. It can, however, replicate in cell cultures from several species, including mouse (fibroblasts and 3T3 cells), hamster, rabbit, sheep, and nonhuman primate.

Clinical signs. MCMV causes asymptomatic infection in adult immunocompetent mice, but experimental inoculation of neonates has caused lethal disease due to multisystemic necrosis and inflammation.

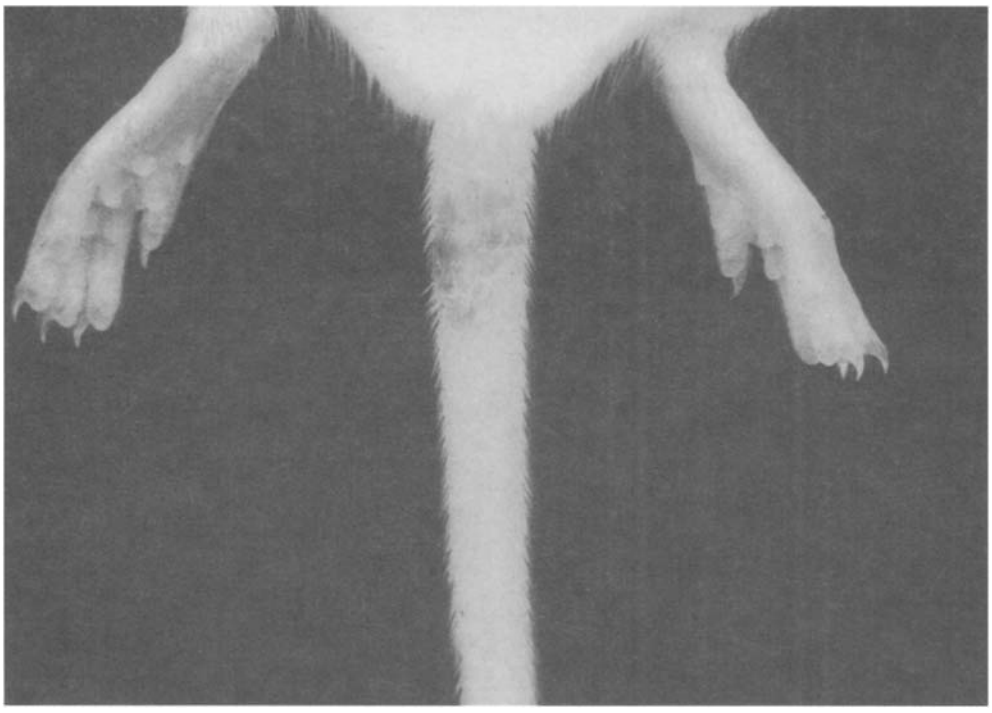

Fig. 17. Vaccination "take" in a mouse inoculated with the IHD-T strain of vaccinia virus. (From Jacoby *et al.*, 1983.)

Epizootiology. The prevalence of MCMV in laboratory mice is unresolved, because infection is clinically silent and serological surveillance is not widely practiced. Wild mice serve as a natural reservoir for infection, which implies that entry of virus into a modern vivarium is most likely to occur from contaminated animal products. Susceptibility to experimental infection varies with age, dose, route, virus strain, and host genotype. Infection can occur in young and adult mice. However, the pathogenicity of MCMV for mice decreases with age. Neonates are highly susceptible to lethal infection, but resistance to disease develops by the time mice are weaned. Immunodeficient mice, however, remain susceptible to pathogenic infection as adults.

Persistence is a central feature of nonlethal infection. Persistently infected mice can excrete virus in saliva, urine, and tears for many months, resulting in horizontal transmission through mouse-to-mouse contact. Virus also can infect prostate, testicle, and pancreas, implicating other modes of excretion. Vertical transmission does not appear to be a common factor in natural infection. Further, maternal immunity protects sucklings from infection.

Pathology. Mouse cytomegalovirus can replicate in many tissues, and viremia commonly occurs. Lesions are not remarkable during natural infection and may be limited to occasional enlarged cells (megalocytosis) containing eosinophilic intranuclear and/or cytoplasmic inclusions associated with lymphoplasmacytic interstitial inflammation, especially in the cervical salivary glands. As noted above, infection in infant mice can induce multisystemic necrosis and inflammation. Persistent infection often affects the salivary glands and pancreas. The persistence of salivary gland infection appears to be dose dependent. There is experimental evidence that MCMV can produce latent infection of B cells, probably T cells as well as aforementioned tissues. Persistent infection may lead to immune complex glomerulonephritis. Latent persistent infection can be reactivated by lymphoproliferative stimuli and by immunosuppression.

Diagnosis. MCMV antigens appear to be weak stimuli for humoral antibody production, which is consistent with the fact that cellular immunity is critical for protection against infection. Neutralizing (NT) antibody titers are low during acute infection and difficult to find during chronic infection. An ELISA has been developed for serological monitoring (Lussier *et al.,* 1987a) and primers for PCR-based diagnosis are available, but neither is widely used because of assumptions that infection has a very low prevalence. Mouse cytomegalovirus can be grown in mouse embryo fibroblasts or 3T3 cells, but cocultivation may be required to rescue latent virus. Detection of enlarged cells with intranuclear inclusions, especially in salivary glands, are diagnostic, if they are present. *In situ* hybridization can be used as an adjunct to routine histopathology.

Differential diagnosis. MCMV infection must be differentiated from infection with mouse thymic virus. The latter virus can produce necrosis and atrophy of thymic and peripheral lymphoid tissue. Lytic lesions of lymphoid tissues are not a hallmark of MCMV. The viruses can also be distinguished from each other serologically. Sialoadenitis with inclusions can occur during infection with polyomavirus. Reovirus 3, mammary tumor virus, and mouse thymic virus can infect the salivary gland.

Prevention and control. Control measures for MCMV have not been established, because it has not been considered an important infection of laboratory mice. Cage-to-cage transmission has not been demonstrated, but horizontal infection from contaminated saliva must be considered. The exclusion of wild mice is essential.

Research complications. Mouse cytomegalovirus can suppress immune responses. Apart from the potential for interfering with immunology research, it can exacerbate the pathogenicity of opportunistic organisms such as *Pseudomonas aeruginosa.*

ii. Mouse thymic virus (MTV) infection (Morse, 1994)

Etiology. MTV is a herpesvirus that is antigenically distinct from MCMV. No suitable *in vitro* method for cultivation has been developed; therefore viral propagation depends on mouse inoculation.

Clinical signs. Natural infections are asymptomatic.

Epizootiology. The prevalence of MTV is thought to be low. Mice can be infected at any age, although lesions develop only in mice infected perinatally. Mice infected as infants or adults can develop persistent infection of the salivary glands lasting several months or more. Excretion of virus in saliva is considered the primary factor in transmission of infection, especially to infant mice. Seroconversion occurs in adults but does not eliminate infection. Infection in neonates may not elicit seroconversion, rendering such mice serologically negative carriers. The mode of infection is obscure, but virus is excreted in saliva, suggesting that transmission from infected dams to neonatal mice occurs by ingestion. MTV also has been isolated from the mammary tissue of a lactating mouse, suggesting the potential for transmission during nursing. Prenatal transmission has not been found.

Pathology. MTV causes severe, diffuse necrosis of the thymus in mice exposed within approximately 1 week after birth, but the severity of thymic and lymph node necrosis can be mouse strain–dependent. Grossly, the thymus is smaller than normal. Infected thymocytes display MTV-positive intranuclear herpetic inclusions. Viral antigen can be demonstrated in the thymus by immunocytochemical staining. Necrosis is followed by granulomatous inflammation and syncytium formation. Necrosis and inflammation can also occur in lymph nodes. Reconstitution of lymphoid organs takes 3–8 weeks.

Diagnosis. Thymic necrosis associated with intranuclear herpetic inclusions is the hallmark lesion. Virus also can be detected by immunohistochemistry. Seroconversion can be detected by ELISA or by IFA. Suspicion of infection in seronegative mice can be tested by inoculation of virus-free neonatal mice with homogenates of salivary gland or with saliva. Inoculated mice should be examined for typical lesions 10–14 days later. PCR methodology or the mouse antibody production (MAP) test can also be used to detect infection.

Differential diagnosis. Reduction of thymus mass can occur in severe mouse coronavirus infection, during epizootic diarrhea of infant mice, or following stress.

Prevention and control. Because MTV induces persistent salivary infection, rederivation or restocking should be considered if infection cannot be tolerated as a research variable.

Research complications. MTV transiently suppresses cellular and humoral immune responses because of its destructive effects on neonatal T lymphocytes.

c. *Parvovirus Infections (Jacoby and Ball-Goodrich, 1995; Jacoby et al., 1996)*

For many years, minute virus of mice (MVM) was recognized as the sole parvovirus of laboratory mice. Recent research has confirmed natural infection with a newly recognized serogroup. The prototype isolate was initially called mouse orphan parvovirus, but has been renamed mouse parvovirus (MPV).

i. Minute virus of mice (MVM) (murine minute virus)

Etiology. MVM is a small (5-kilobase) single-stranded DNA virus. The prototypic strain is designated MVM(p). An allotropic variant with immunosuppressive properties *in vitro* also has been identified and is named MVM(i). The genome encodes two nonstructural proteins, NS-1 and NS-2, that are highly conserved among the rodent parvoviruses and account for prominent cross-reactivity in generic serological assays. The viral capsid proteins, VP-1 and VP-2, are virus-specific and form the basis for serological differentiation of MVM from mouse parvovirus (MPV). MVM has a broad *in vitro* host range. It replicates in monolayer cultures of mouse fibroblasts (A9 cells), C6 rat glial cells, SV40 (simian virus 40)-transformed human newborn kidney (324K cells), T cell lymphomas (EL4), and rat or mouse embryo cells, producing cytopathic effects that can include the development of intranuclear inclusions.

Clinical signs. Natural infections are asymptomatic. Neonatal mice of some inbred strains are susceptible to lethal renal and/or intestinal hemorrhage during experimental MVM(i) infection, but this syndrome has not been reported in natural outbreaks.

Epizootiology. MVM is perceived as a common virus of mice. Its prevalence in mouse colonies surveyed during the 1970s and 1980s was reported to be approximately 30–90%. A recent survey of major biomedical research centers revealed an overall prevalence of parvovirus infection of about 25% among specific pathogen-free mice and 40% among conventionally housed mice (Jacoby and Lindsey, 1997). However, the earlier data did not account for MPV infections, which are now known to have been present during those decades, and the recent survey did not report results for MVM and MPV separately. Therefore, the true prevalence of MVM (as opposed to MPV) is unclear. The recent development of a strain-specific ELISA should resolve this issue.

MVM is highly infectious for the mouse, its only known natural host. Virus can infect the gastrointestinal track and is excreted in feces and urine. The resistance of rodent parvoviruses to environmental inactivation increases the risks of transmission after virus is excreted. Therefore, contamination of caging, bedding, food, and clothing must be considered a risk for the spread of infection. Transmission occurs by oronasal exposure, but viral contamination of biologicals used for experimental inoculation, such as transplantable tumors, also can be a source of infection. Continuous contact exposure to infected animals or soiled bedding usually induces a humoral immune response within 3 weeks, but limited exposure may delay seroconversion. Young mice in enzootically infected colonies are protected by maternal antibody, but actively acquired immunity develops from infection sustained after the decay of maternal immunity. MVM, in contrast to MPV, is not thought to cause persistent infection; infection in immunocompetent adult mice usually lasts less than 3 weeks (Smith, 1983b; Smith and Paturzo, 1988). Infection appears to last less than 1 month even in oronasally inoculated neonatal mice. Although MVM has not been studied in immunodeficient mice, one should assume that infection will be prolonged in such mice. There is no evidence that MVM is transmitted *in utero.*

Pathology. Natural infections or experimental inoculation of adult mice appears to be nonpathogenic, although low-level cytolysis *in situ* cannot be excluded. Contact-exposed neonates have been reported to develop cerebellar lesions, but these are very rare. Experimental infection of neonatal BALB/c, SWR, SJL, CBA, and C3H mice with MVM(i) can cause renal hemorrhage and infarction (Brownstein *et al.,* 1991). DBA/2 mice also developed intestinal hemorrhages and accelerated involution of hepatic hematopoiesis. C57BL/6 neonates are resistant to vascular disease. This lesion has been attributed to viral infection of endothelium.

Diagnosis. ELISA serology is the primary method to detect infection. Additionally, MVM can be differentiated from MPV using virus-specific VP-2 antigens (L. J. Ball-Goodrich, unpublished results, 2000). MVM infection also can be detected by

PCR, *in situ* hybridization, and immunohistochemistry. Although the most commonly used molecular assay is PCR amplification of a conserved portion of NS-1, it does not differentiate MVM from MPV. However, virus-specific PCR assays that amplify gene segments within the capsid protein genes also are available (Besselsen, 1998). MVM can be isolated from spleen, kidney, intestine, and other tissues by inoculation of the C6 rat glial cell line. It also can be detected by the mouse antibody production test.

Prevention and control. Because MVM does not persist in immunocompetent mice, control and elimination should exploit quarantine combined with thorough disinfection of the environment, because parvoviruses are resistant to environmental inactivation. However, there are no published reports confirming the success of this strategy. Additionally, reliance on quarantine presumes that MPV infection, which can be persistent in adult immunocompetent mice, has been ruled out. If the identification of the virus remains problematic, a more stringent approach such as cesarean rederivation or embryo transfer may be preferable. Prevention of MVM infection depends on strict barrier husbandry and regular surveillance of mice and mouse products destined for use *in vivo.*

Research complications. MVM contamination of transplantable neoplasms is quite common; therefore, infection can be introduced to a colony through inoculation of contaminated cell lines. Failures to establish long-term cell cultures from infected mice or a low incidence of tumor "takes" should alert researchers to the possibility of MVM contamination. MVM(i) has the potential to inhibit the generation of cytotoxic T cells in mixed lymphocyte cultures.

ii. Mouse parvovirus (MPV)

Etiology. During the mid-1980s, serological testing revealed a murine parvovirus that was antigenically distinct from MVM (McKisic *et al.,* 1993). The virus was isolated following its detection as a lymphocytotropic contaminant in *in vitro* assays for cellular immunity. The virus grew lytically in a $CD8^+$ T cell clone designated L3 and inhibited the proliferation of cloned T cells stimulated with antigen or interleukin 2 (IL-2). Additional isolates confirmed that these viruses are antigenically distinct from MVM. Thus, they constitute a second and distinct serogroup of murine parvoviruses. Molecular analysis of MPV indicates that regions encoding the NS proteins are similar to those of MVM, a finding that accounts for cross-reactivity between the viruses in generic serological tests. However, they differ significantly in regions encoding the capsid proteins, accounting for their antigenic specificity (Ball-Goodrich and Johnson, 1994). The prototype isolate was first called an "orphan" parvovirus of mice because its biology and significance were obscure, but it has subsequently been named mouse parvovirus (MPV). MPV is very difficult to grow *in vitro.* Immortalized T cells (L3) are the only cells found thus far to support replication of MPV.

Clinical signs. MPV infection is clinically silent in infant mice and adult immunocompetent or immunodeficient mice.

Epizootiology. Serologic evidence strongly suggests that MPV causes natural infection only in mice. Infection has circulated in mouse colonies in the United States for at least 30 years. Retrospective testing indicated that the prevalence of MPV approached 40% in the early 1970s, whereas only 7% of tested sera contained MVM antibody. *In situ* hybridization has identified the small intestine as a site of viral entry and early replication, but respiratory infection cannot be excluded. Based on these findings and initial transmission studies, MPV is thought to be transmitted primarily by fecal excretion and ingestion of contaminated material. There is no evidence of prenatal transmission. Initial studies indicated that humoral (e.g., passively or maternally acquired) immunity can protect against MPV infection. However, immunity to MVM may not confer cross-immunity to MPV (Hansen *et al.,* 1999).

MPV causes persistent infection in infant and adult mice, a property that differentiates it from MVM. *In situ* hybridization studies detected viral DNA in the lymph nodes of experimentally infected adult mice for at least 9 weeks. Furthermore, infection has been transmitted by adults to naive cagemates intermittently for up to 6 weeks (Smith *et al.,* 1993).

Pathology. MPV appears to enter through the intestinal mucosa, which is a site of early virus replication (Fig. 18). Acute infection is widespread but mild, involving lung, kidney, liver, and lymphoid organs. Histological lesions are not discernible, despite the potential for cytolysis during parvovirus replication. Lymphocytotropism is a characteristic of acute and persistent MPV infection in infant and adult mice. During acute infection virus is dispersed within lymph nodes, but during persistent infection virus localizes in germinal centers (Fig. 19).

Diagnosis. Because infected mice do not manifest signs or lesions and the virus is very difficult to propagate in cell culture, detection and diagnosis rely on serology and molecular methods. A recently developed MPV-specific ELISA that uses MPV VP-2 as antigen is a sensitive and specific assay that differentiates MPV from MVM (L. J. Ball-Goodrich, unpublished results, 2001). The MAP test also can be used to detect parvovirus infections but is relatively time-consuming and expensive.

As noted for MVM, a generic PCR assay for murine parvoviruses, using conserved primer sequences that are conserved among murine parvoviruses, can be used as a screening test. PCR also can be used to detect MPV-specific sequences in the VP-2 gene. Although diagnostic PCR is sensitive and specific, it is effective only in actively infected animals and requires ac-

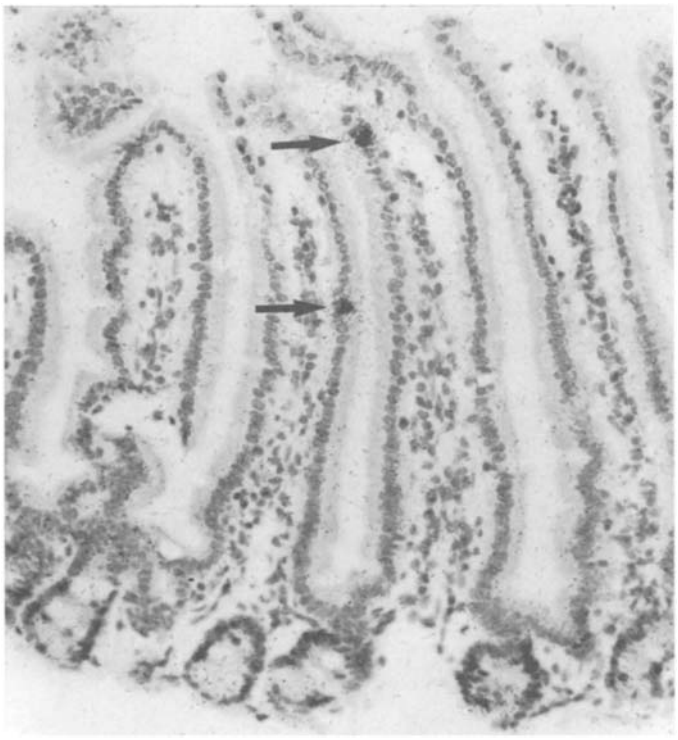

Fig. 18. Mouse parvovirus (arrows) in the intestine after oronasal inoculation of an adult mouse. *In situ* hybridization.

cess to tissues that are usually obtained at necropsy. Therefore, its primary value is to confirm serological results.

Differential diagnosis. MPV infection must be differentiated from MVM infection. Because both viruses are enterotropic and lymphocytotropic, serology and molecular hybridization must be used to distinguish between them.

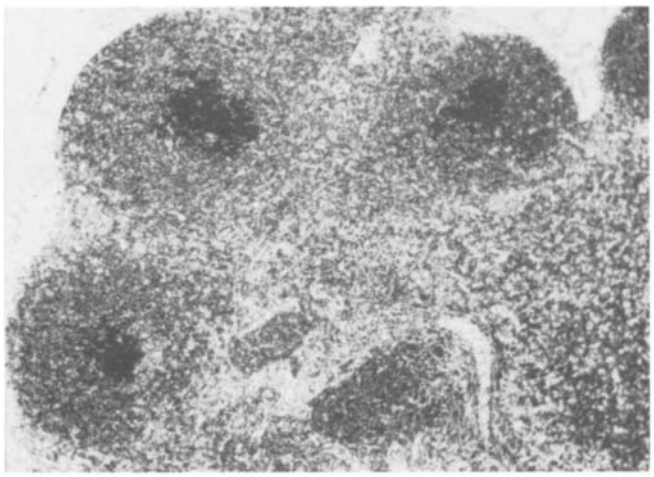

Fig. 19. Mouse parvovirus in the mesenteric lymph node of a persistently infected mouse. *In situ* hybridization.

Prevention and control. The persistence of MPV in individual mice, its potential for provoking immune dysfunction, and the resistance of murine parvoviruses to environmental inactivation favor active control and prevention of MPV infection. Quarantine of infected rooms is appropriate. Elimination (depopulation) of infected mice should be considered if they are an immediate threat to experimental or breeding colonies and can be replaced. For mice that are not easily replaced, virus persistence in the absence of transplacental transmission favors cesarean rederivation or embryo transfer as relatively rapid options to eliminate infection. Control of infection also should include environmental decontamination. Chemical disinfection of suspect animal rooms and heat sterilization of caging and other housing equipment are prudent steps. Prevention is based on sound serological monitoring of mice and surveillance of biologicals destined for inoculation of mice.

Research complications. Murine parvoviruses can distort biological responses that depend on cell proliferation. For MPV, such effects are seen on immune function and include augmentation or suppression of humoral and cellular immune responses.

d. Mouse Adenovirus Infection (Richter, 1986; Porterfield and Richter, 1994a)

Etiology. Adenoviruses are nonenveloped DNA viruses that produce intranuclear inclusions *in vitro* and *in vivo*. Two adenovirus strains have been associated with mice: MAdV-1 (FL) and MAdV-2 (K87). Both strains replicate in mouse kidney tissue culture but are antigenically distinct.

Clinical signs. MAdV-1 can cause severe clinical disease after experimental inoculation of infant mice. Signs include scruffiness, lethargy, stunted growth, and often death within 10 days. MAdV-2 virus is enterotropic and appears to be responsible for virtually all naturally occurring infections. Infection is usually asymptomatic in immunocompetent mice, with the possible exception of transient runting among infant mice.

Epizootiology. The prevalence of adenovirus infection in mouse colonies is not well documented but appears to be low. Transmission occurs by ingestion. Adult mice experimentally infected with MAdV-1 may remain persistently infected and excrete virus in the urine for prolonged periods. Adult mice experimentally infected with MAdV-2 excrete virus in feces for at least 3 weeks but eventually recover. Athymic mice can shed MAdV-2 for at least 6 weeks and episodically for at least 6 months.

Pathology. Infection with MAdV-1 causes multisystemic disease characterized by necrosis. Infant mice are especially susceptible to rapidly fatal infection characterized by necrosis of

brown fat, myocardium, adrenal cortex, salivary gland, and kidney, including the development of intranuclear inclusions. More mature mice usually develop subclinical infection leading to seroconversion; however, athymic mice can develop intestinal hemorrhage amd wasting. Infection with MAdV-2 produces amphophilic, intranuclear inclusions in intestinal epithelium, especially in the distal small intestine and cecum (Fig. 20). Inclusions are easier to detect in infant mice than in adults.

Diagnosis. Although MAdV strains can be isolated in tissue culture, routine diagnosis depends on detection of infection by serological assay and/or demonstration of adenoviral inclusions, most commonly in the intestinal mucosa. An immunofluoresence assay and an ELISA are available for serological surveillance (Smith *et al.,* 1986; Lussier *et al.,* 1987b). Cross-neutralization tests have revealed that antiserum to MAdV-2 neutralizes both strains, but antiserum to MAdV-1 neutralizes MAdV-2 weakly at best. Therefore, MAdV-2 antigen should be used for the serological detection of adenovirus infection irrespective of the assay employed. MAdV also can be detected by PCR (S. R. Compton, personal communication, 2001).

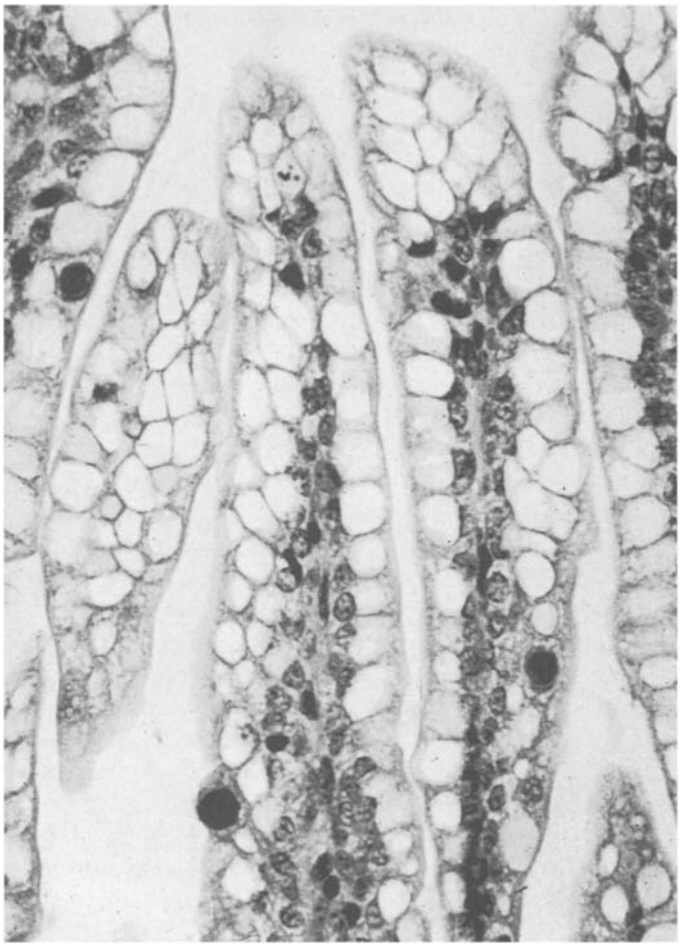

Fig. 20. Intranuclear adenoviral inclusions in mouse intestine. (Courtesy of Dr. S. W. Barthold.)

Differential diagnosis. The intranuclear adenoviral inclusions in intestinal epithelium are pathognomonic and differentiate MAdV-2 infection from other known viral infections of mice.

Prevention and control. Prevention requires serological monitoring of mice and exmination for passenger viruses of animal products such as transplantable tumors. Because MAdV-2 infection appears to be transient in individual mice, segregation of infected colonies may be effective for control. However, rederivation coupled with subsequent barrier housing is a more conservative approach.

Research complications. MAdV infection is unlikely to affect research using immunocompetent mice. However, it has the potential for pathogenicity in immunodeficient mice.

e. Papovavirus Infections (Shah and Christian, 1986)

Mice can incur natural infection with two papovaviruses: polyomavirus and K virus.

i. Polyomavirus (Orcutt, 1994)

Etiology. Polyomavirus is a small DNA virus. It is highly antigenic in adult mice but induces multiple types of tumors in mice infected as neonates. Its primary importance stems from use in murine models of experimental oncogenesis, with natural infection being rare.

Clinical signs. Natural infections in immunocompetent mice are usually asymptomatic. However, tumor induction, neurological disease, and wasting can occur in immunodeficient mice (McCance *et al.,* 1983; Sebesteny *et al.,* 1980).

Epizootiology. Modern husbandry and health care have essentially eliminated natural exposure in laboratory mice. Because infection can be introduced inadvertently and is highly contagious, some additional features are highlighted here. Inoculation of neonatal mice with contaminated biologicals or cell cultures is a potential source of entry and spread. Once infection is established, virus is shed in urine, feces, and saliva, followed by intranasal exposure of other mice. Thus airborne dissemination results primarily from contaminated feed and bedding. Intrauterine infection also can occur, and persistent renal infection, contracted neonatally, can be reactivated during pregnancy. Exposure of neonatal mice also can result in viremic dissemination and high mortality. Additionally, survivors can be persistently infected and excrete virus from lung and kidney for months. Infection of breeding-age mice usually results in rapid

clearance of virus and protection of offspring by maternally derived antibodies. However, PCR has revealed infection lasting up to 5 months in CBA mice inoculated with virus as adults (Berke and Dalianis, 1993). Polyomavirus infection can persist in adult immunodeficient mice.

Pathology. Polyomavirus-induced tumors are primarily a laboratory phenomenon. Experimental inoculation of neonatal mice can produce viremia and acute, lethal disease. Tumors appear 2–12 months after inoculation of surviving mice, and in most strains the salivary glands are prevalent sites for tumor development. However, tumors can occur at other sites, especially in skin adnexa, the upper gastrointestinal tract, and the kidneys. The location of tumors varies with virus strain and, to some extent, with the route of inoculation. Athymic mice can develop cytolytic and inflammatory lesions, followed by multisystemic tumor formation. Intranuclear inclusions may be present in cytolytic lesions. Demyelinating disease has been reported in experimentally inoculated athymic mice (see references under "Clinical Signs," above), and myeloproliferative disease has been reported in experimentally inoculated SCID mice (Szomolanyi-Tsuda *et al.,* 1994).

Diagnosis. Virus can be isolated in mouse fibroblast cell lines, but infection is ordinarily detected serologically by ELISA (Broeders *et al.,* 1994). Additionally, PCR and immunohistochemistry can be used (S. R. Compton, personal communication, 2001).

Differential diagnosis. Wasting in athymic mice can be caused by other infectious agents, including coronaviruses, Sendai virus, and *Pneumocystis carinii.* Intranuclear inclusions can occur in infections caused by mouse adenovirus, mouse cytomegalovirus, and K virus.

Prevention and control. Control depends on elimination of infected mice and material, together with prevention of airborne spread. Tumor and cell lines destined for mouse inoculation should be tested for polyomavirus by the mouse antibody production test or molecular diagnostics.

Research complications. Polyomavirus infection can affect experiments by inadvertent contamination of cell lines or transplantable tumors, leading to infection of inoculated mice and the potential for epizootic spread.

ii. K virus infection (Parker and Richter, 1982; Porterfield and Richter, 1994b)

K virus is a papovavirus of mice that has historical importance but is of little consequence to contemporary mouse colonies. Oral inoculation of neonatal mice results in initial infection of capillary endothelium in the intestine, followed by viremic spread. Vascular endothelium is the primary target in affected tissues, which often include lung, liver, spleen, and adrenal glands. Dyspnea occurs from pulmonary infection because of edema and hemorrhage. Infection of immunocompetent adult mice is asymptomatic and results in a vigorous immune response. However, both adults and infant mice develop persistent infection. Additionally, infection of athymic mice can lead to clinical signs and lesions akin to those described for neonatally inoculated mice. Gross lesions are limited to pulmonary hemorrhage and edema. Histologically, intranuclear inclusions, which are visualized more easily using immunohistochemistry, are present in vascular endothelium of infected tissues. Mild hepatitis with hepatocytic degeneration also may develop. Infection can be detected by ELISA serology or PCR. Prevention and control measures are similar to those described for polyomavirus, except that precautions against airborne transmission are not required.

f. Lactate Dehydrogenase–Elevating Virus (LDV) Infection (Brinton, 1982, 1986)

Etiology. LDV is a togavirus specific to mice that increases the concentration of several serum enzymes, most notably lactate dehydrogenase (LDH).

Clinical signs. Infection is typically asymptomatic. However, poliomyelitis has occurred in immunosuppressed C58 and AKR mice inoculated with LDV.

Epizootiology. The prevalence of LDV infection is thought to be low. The primary mode of mouse-to-mouse transmission is mechanical transfer from aggressive behavior (e.g., bite wounds). Inoculation of mice with contaminated animal products such as cell lines, transplantable tumors, or serum is probably the most common source of induced infection. It is important to note, with respect to mechanical transmission, that infection induces lifelong viremia. Natural transmission between cagemates or between mother and young is rare even though infected mice may excrete virus in feces, urine, milk, and probably saliva.

Pathology. Viremia peaks within 1 day after inoculation, then persists at a diminished level. The elevation of enzyme levels in blood is thought to result primarily from viral interference with clearance functions of the reticuloendothelial system. No lesions are seen in naturally infected mice. The only significant lesion thus far associated with experimental infection is polioencephalitis in immunosuppressed C58 and AKR mice. Mild leptomeningitis and myelitis have been reported in C57BL/6 mice. T cell–dependent areas of thymus and peripheral lymphoid tissue may undergo mild necrosis early in experimental

infection. Immune complex glomerular disease is not a significant complication of LDV infection, despite the propensity of the virus to form immune complexes.

Diagnosis. Plasma LDH levels are elevated, a response that is used to detect and titrate LDV infectivity. Of the five isoenzymes of LDH-V in mouse plasma, only LDV is elevated. SJL/J mice in particular show spectacular increases in LDH levels (15–20 times normal), a response controlled by a recessive somatic gene. LDV is detected by measuring LDH levels in mouse plasma before and 4 days after inoculation of specific pathogen–free (SPF) mice with suspect material. It is important to use nonhemolysed samples because hemolysis will produce falsely elevated readings. Plasma enzyme levels are measured in conventional units/ml, 1 conventional unit being equivalent to 0.5 International Units (IU). Normal plasma levels are 400–800 IU, whereas in LDV infection, levels as high as 7000 IU can occur. LDV also interferes with the clearance of other serum enzymes and results in their elevation in serum.

Infection provokes a modest humoral antibody response, but it is difficult to detect because of formation of virus–antibody immune complexes. Molecular diagnostics also can be used to diagnose infection in mouse tissues and serum and in cell cultures. However, inhibitory factors in cells and serum may cause false negative results in PCR testing, so appropriate quality control measures are essential if this method is used (Lipman and Henderson, 2000).

Prevention and control. Transplantable tumors have been a common source of LDV historically. Therefore, tumors or cell lines destined for mouse inoculation should be monitored for LDV contamination. Although LDV can infect tumor cells, it does not replicate in them. Therefore, one can attempt to free tumors of virus by passaging them several times in rodents nonpermissive to LDV (e.g., rats) before repassaging them in mice.

Research complications. LDV has numerous potential effects on immunological function. It may reduce autoantibody production, cause transient thymic necrosis and lymphopenia, suppress cell-mediated immune responses, and enhance or suppress tumor growth.

g. *Lymphocytic Choriomeningitis Virus (LCMV) Infection (Lehmann-Grube, 1982; Lindsey et al., 1991b)*

Etiology. LCMV is a single-stranded, enveloped RNA virus that buds from the cell membrane without cytolysis. Its name is derived from the immune-mediated inflammation resulting from the intracerebral inoculation of virus into immunologically competent mice. Virus strains are closely related antigenically but vary in their rate of replication, tissue tropism, pathogenicity, and immunogenicity. Furthermore, these properties can be modulated by passage *in vivo* or *in vitro*. Neurotropic and viscerotropic strains have been used extensively to develop and study mouse models of virus-induced immune injury and to reveal fundamental mechanisms of immune recognition (Doherty, 1997). LCMV can infect insect cells as well as mammalian cells and can persistently infect naturally exposed mice and cultured cells.

Clinical signs. Clinical signs of LCMV infection vary with age and strain of mouse, route of inoculation, and strain of virus. Natural infection in immunocompetent adult mice is usually self-limiting and asymptomatic. However, four basic patterns of clinical disease are recognized from study of experimentally induced infection (Fig. 21). (1) The cerebral form is characterized by illness in immunocompetent mice beginning 5–6 days after intracerebral inoculation of virus. Sudden death may result or subacute illness associated with one or more of the following signs may develop: ruffled fur, hunched posture, motionlessness, and neurological deficits. Mice suspended by the tail display coarse tremors of the head and extremities, culminating in clonic convulsions and tonic extension of the hindlegs. Spontaneous convulsions also can occur. Animals usually die or recover in several days. (2) A visceral form can occur in adult mice inoculated by peripheral routes with viscerotropic strains. It can be asymptomatic or lead to clinical signs, including ruffled fur, conjunctivitis, ascites, somnolescence, and death. If mice survive, recovery may take several weeks. (3) Runting and death from LCMV infection may occur in neonatally infected suckling mice and can lead to transient illness or to death. Clinical signs are nonspecific, recovery is slow, and survivors may remain runted. This early form of disease is attributed to endocrine dysfunction caused by LCMV infection. (4) Late-onset disease can occur in previously asymptomatic carrier mice that develop immune complex glomerulonephritis. It is usually the result of prenatal or neonatal infection and occurs in persistently infected mice when they are 9–12 months old. Clinical signs are nonspecific and include ruffled fur, hunched posture, weight loss, proteinuria, and ascites.

Epizootiology. LCMV is distributed widely in wild mice in North and South America and in Europe and has recently been found in Australia (Smith *et al.,* 1993). Among common laboratory species, mice, hamsters, guinea pigs, and nonhuman primates are susceptible to infection, but only the mouse and the hamster are known to transmit virus. LCMV infection is not prevalent in laboratory mice produced and maintained in modern quarters. Infection is usually introduced through inoculation of virus-infected biologicals, such as transplantable tumors, or by feral mice. Wild mice are a natural reservoir of infection and a potential threat to research colonies if they gain entry inadvertently. Carrier mice usually develop as a result of asymptomatic prenatal or neonatal infection, which induces tolerance to the virus. Such mice can have persistently high con-

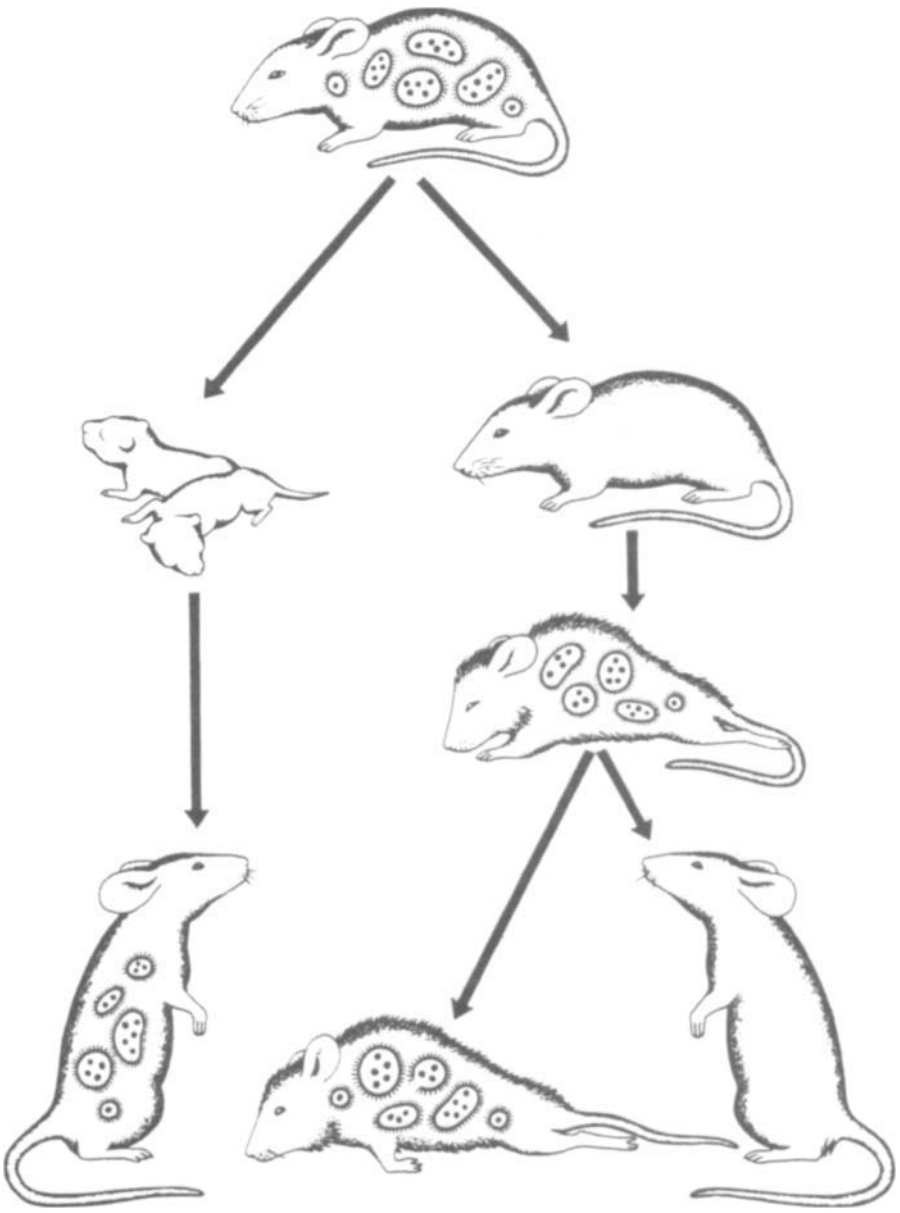

Fig. 21. Basic phenomena associated with LCMV infection in mice. (From Lehman-Grube, 1982.)

centrations of virus in many organs, thereby facilitating virus excretion in saliva, nasal secretions, and urine. Persistently infected neonates usually reach breeding age and can perpetuate infection in a breeding colony. Thus introduction of a single LCMV carrier mouse to a breeding colony can eventually result in a high prevalence of persistently infected mice. Infection in adult mice, by contrast, is often acute because of the onset of effective immunity, and the spread of virus is halted. Horizontal spread of infection is enhanced by close contact, but rapid horizontal spread is not characteristic. Mice can transmit LCMV to hamsters, which can remain viremic and "viruric for many months even if they contract infection as adults. Infected hamsters can transmit virus to other hamsters and mice and are the primary source of human LCMV infection (see Chapter 5). Infected mice are not a significant source of human exposure. However, persistent infection in immunodeficient mice may carry greater risks for viral excretion and zoonotic transmission.

Pathology. LCMV disease is a prototype for virus-induced, T lymphocyte–mediated immune injury and for immune complex disease. However, lesions comparable to experimentally induced disease are rare during natural infection. Intracerebral inoculation of virus into immunocompetent adult mice can induce nonsuppurative leptomeningitis, choroiditis,

and focal perivascular lymphocytic infiltrates. Host tissues are damaged during the course of the cellular immune response to the virus. The character of visceral lesions depends on virus strain and mouse strain; the ratio of cytolytic to proliferative responses in lymphoid organs is mouse strain–dependent. In severe infection, nonsuppurative inflammation can occur in many tissues. The severity of accompanying cytolytic lesions seems to parallel the intensity of cellular immunity. Liver lesions can include hepatocytic necrosis accompanied by nodular infiltrates of lymphoid cells and Kupffer's cells, activated sinusoidal endothelium, an occasional granulocyte or megakaryocyte, and fatty metamorphosis. Cytolysis, cell proliferation, and fibrinoid necrosis can develop in lymphoid organs. Necrosis of cortical thymocytes can lead to thymic involution. Lesions of late-onset disease are characterized by formation of immune complexes and associated inflammation. Renal glomeruli and the choroid plexus are most severely affected, but complexes may also be trapped in synovial membranes, blood vessel walls, and skin. Lymphoid nodules can form in various organs. Lesions associated with early deaths in neonatally infected mice have not been thoroughly described but include hepatic necrosis.

The lesions of acute and persistent LCMV infection reflect separate immunopathological processes. In adult mice with acute LCMV infection, virus multiplies in B cells and macrophages, whereas T cells are resistant. Internal viral epitopes induce humoral immune responses, but surface epitopes elicit cell-mediated immunity and neutralizing antibodies. Thus elimination of virus and virus-associated immunological injury are both T cell–mediated. This apparent paradox has been explained by the view that prompt cellular immunity limits viral replication and leads to host survival, whereas slower cellular immune responses permit viral spread and increase the number of virus-infected target cells subject to attack once immunity is fully developed. Antibody can be detected by 1 week after infection but does not play a significant role in eliciting acute disease. Lesions of LCMV infection appear to develop from direct T cell–mediated damage to virus-infected cells and may involve humoral factors released from immune effector T cells. LCMV also can suppress humoral and cellular immunity in acutely infected mice.

Persistent infection commonly evolves from exposure early in pregnancy, and virus has been demonstrated in the ovaries of carrier mice. Prenatal or neonatal infection induces immunological tolerance to LCMV, which can then replicate to high titer in many tissues. Nevertheless, persistently infected mice develop humoral antibody to LCMV. Antibody can complex with persistent virus to elicit complement-dependent inflammation in small vessels. Immune complex glomerulonephritis exemplifies this process, as noted above.

Diagnosis. LCMV infection can be diagnosed serologically by IFA or ELISA tests (Homberger *et al.,* 1995). Whereas immunocompetent adult mice will normally seroconvert after exposure, carrier mice may develop poor humoral immune responses. Therefore, testing must avoid false negative results. Employment of adult contact sentinel mice is a useful strategy for detecting LCMV infection by seroconversion. Alternatively, small blood samples can be collected from persistently infected live suspects, which are often viremic, and used to inoculate cultured cells or adult and neonatal mice. Intracerebral inoculation of LCMV-positive tissues should elicit neurological signs in adult mice within 10 days, whereas infant mice should remain asmptomatic. Histological examination of brains from affected adults may reveal nonsuppurative inflammation, but lesions may be minimal in mice infected with viscerotropic isolates. Immunohistochemistry can be used to detect viral antigen in brains of suckling and adult mice. Intraperitoneal inoculation of adult mice may yield short-lived infection with seroconversion, i.e., the mouse antibody protection test. A reverse transcriptase–polymerase chain reaction (RT-PCR) assay also is available (Park *et al.,* 1997). Virus can be grown and quantified in several continuous cell lines, including mouse neuroblastoma (N-18) cells, BHK-21 cells, and L cells. Application of immunofluorescence staining to detect LCMV antigen in inoculated cultured cells yields results more quickly than animal inoculation. Of course, all diagnostic procedures involving potential contact with live virus should be carried out under strict containment conditions to avoid infection of laboratory personnel. The use of *in vitro* detection has the added advantage, in this regard, of reducing biohazardous exposure and the use of live animals for testing.

Differential diagnosis. Neurological signs must be differentiated from those due to mouse hepatitis virus, mouse encephalomyelitis virus, and meningoencephalitis from metastatic bacterial infection. Trauma, neoplasia, and toxicities also must be ruled out in neurological disease with low prevalence. Late-onset disease is associated with characteristic renal lesions, including deposition of viral antigen in tissues. Early-onset disease must be differentiated from other causes of early mortality, such as mouse hepatitis virus, ectromelia virus, reovirus 3 infection, Tyzzer's disease, or husbandry-related insults.

Prevention and control. Adequate safeguards for procurement and testing of animals and animal products are essential to prevent entry. Because mouse-to-mouse spread is slow, selective testing and culling for seropositive or carrier mice is possible. If mice are easily replaced, however, depopulation is a safer and more reliable option. Valuable stock can be rederived, but progeny must be tested to preclude *in utero* transmission. Because infected hamsters can excrete large quantities of virus, exposed hamsters should be destroyed and hamsters should not be housed with mice. LCMV can be transmitted to human beings, who can contract severe CNS disease. More frequently, human infection resembles influenza or is asymptomatic. The zoonotic potential of LCMV infection makes it especially im-

portant to detect and eliminate carrier animals and other potentially contaminated sources, such as cell cultures, transplantable neoplasms, and vaccines to prevent human exposure. Serum banking and periodic serological testing of high-risk human populations, such as those working with LCMV experimentally, is recommended.

Research complications. LCMV may stimulate or suppress immunological responses *in vivo* and *in vitro,* and it can replicate in cells used as targets or effectors for immunological studies. Introduction of immune cells to a carrier animal may elicit an immunopathological response. Immune complex disease can complicate long-term experiments and morphological interpretations. Illness and death in mice and zoonotic risks to humans are obvious research-related hazards.

h. Sendai Virus Infection (Parker and Richter, 1982; Brownstein, 1986)

Etiology. Sendai virus (SV) is a paramyxovirus that is antigenically related to human parainfluenza virus 1. Viral particles are pleomorphic, contain single-stranded RNA, and have a lipid solvent–sensitive envelope that contains glycoproteins with hemagglutinating, neuraminidase, and cell fusion properties. SV grows well on embryonated hens' eggs and in several mammalian cell lines (e.g., monkey kidney, baby hamster kidney [BHK-21], and mouse fibroblast [L]). Virus replicates in the cytoplasm and by budding through cell outer membranes.

Clinical signs. Susceptible adult mice often assume a hunched position and have an erect hair coat. Rapid weight loss and dyspnea occur, and there may be chattering sounds and crusting of the eyes. Although highly susceptible adults may die, lethal infection is more common in suckling mice. Sex differences in susceptibility have not been found. Genetically resistant mice usually have asymptomatic infection, especially if they are otherwise in good health. Athymic mice and immunosuppressed mice are at high risk and can develop a wasting syndrome. However, they develop illness later than their immunocompetent counterparts. Opportunistic infections can complicate the clinical presentation. For example, secondary bacterial infections of the ear can cause vestibular signs.

Epizootiology. The prevalence of SV infection, once among the highest for infectious agents of the mouse, has decreased sharply in recent years (Jacoby and Lindsey, 1997). This is probably attributable to several factors, including improvements in housing systems, health monitoring, and production standards for mice.

SV is transmitted by aerosol and is highly infectious. Morbidity in infected colonies is commonly 100%, and mortality can vary from 0 to 100%, partly because strains of mice vary greatly in their susceptibility to lethal SV infection. For example, C57BL/6 mice are highly resistant to clinically apparent infection, whereas DBA/2 mice are highly susceptible. Aerogenic infection is promoted by high relative humidity and by low air turnover. Prenatal infection does not occur. Enzootic infection is commonly detected in postweaned mice (5–7 weeks old) and is associated with seroconversion within 7–14 days and the termination of infection. Therefore, entrenched infection is perpetuated by the introduction of susceptible animals. There is no evidence for persistent infection in imunocompetent mice, but prolonged infection is common in immunodeficient mice. Maternally acquired immunity protects young mice from infection, and actively acquired immunity is thought to be long-lived. Rats, hamsters, and guinea pigs also are susceptible to SV infection. Therefore, bidirectional cross-infection is a risk during outbreaks.

Pathology. Viral replication during natural infection is nominally restricted to the respiratory tract and peaks by the first week after infection. Gross lesions feature partial to complete consolidation of the lungs (Fig. 22). Individual lobes are meaty and plum-colored, and the cut surface may exude a frothy serosanguinous fluid. Pleural adhesions or lung abscesses caused by secondary bacterial infection are seen occasionally, and fluid may accumulate in the pleural and pericardial cavities.

SV targets airway epithelium and type II pneumocytes. Type I pneuomocytes are less severely affected. Histologically,

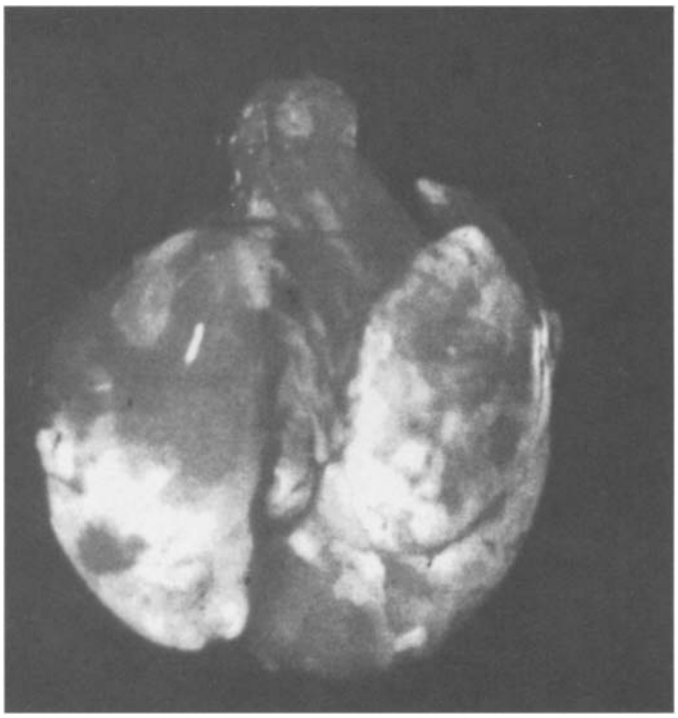

Fig. 22. Pulmonary consolidation resulting from Sendai viral pneumonia.

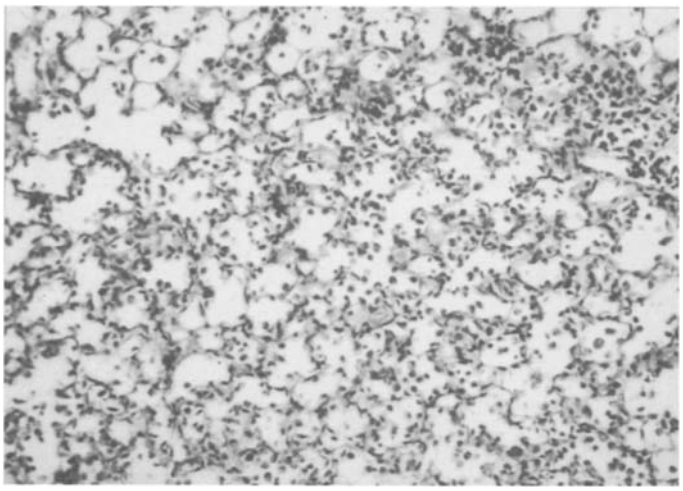

Fig. 23. Interstitial pneumonia caused by Sendai virus.

the pattern of pneumonia is influenced by mouse genotype. Susceptible mice usually have significant bronchopneumonia and interstitial penumonia, whereas the interstitial component may be less prominent in resistant mice (Fig. 23). Typical changes begin with inflammatory edema of bronchial lamina propria, which may extend to alveolar ducts, alveoli, and perivascular spaces. Necrosis and exfoliation of bronchial epithelium ensue, frequently in a segmental pattern (Fig. 24). Alveolar epithelium also may desquamate, especially in severe disease, and necrotic cell debris and inflammatory cells can accumulate in airways and alveolar spaces. Alveolar septae are usually infiltrated by leukocytes to produce interstitial pneumonia. Lymphoid cells also invade epibronchial and perivascular spaces. The lymphocytic response to SV infection reflects the fact that cellular immunity contributes both to lesions and to recovery. This apparent paradox may be attributable an immunopathological

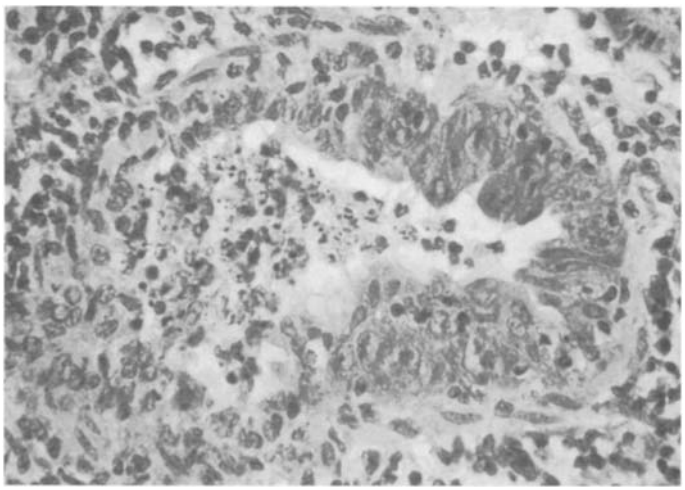

Fig. 24. Bronchopneumonia caused by Sendai virus.

mechanism in the development of Sendai viral pneumonia. Local immunoglobulin synthesis by infiltrating cells also occurs. The extent of inflammatory cell infiltration corresponds to the level of genetic resistance expressed by the infected host, with susceptible hosts mounting a more florid response than resistant hosts. Additionally, strain-related differences in the severity of infection may reflect differences in airway mucociliary transport. Multinucleated syncytia are occasionally seen in affected sucklings, and inclusion bodies have been reported in infected athymic mice.

Regeneration and repair begin shortly after the lytic phase and are characterized by hyperplasia and squamous metaplasia of bronchial epithelium, which may extend into alveolar septae. Proliferation of cuboidal epithelium may give terminal bronchioles an adenomatoid appearance. Repair of damaged lungs is relatively complete in surviving mice, but lymphocytic infiltrates, foci of atypical epithelium, and mild scarring can persist. Acute phase lesions are prolonged in immunodeficient mice, which can lead to wasting and death. Aged mice also have a prolonged recovery phase accompanied by focal pulmonary fibrosis (Jacoby *et al.,* 1994).

Diagnosis. Clinical signs of respiratory distress are highly suggestive of SV infection, especially among infant mice or adults of genetically susceptible strains. However, ELISA or IFA serology is an effective means to detect infection in all strains of immunocompetent mice (Wan *et al.,* 1995). Antibody can be detected by 7 days postinfection. Repeated serologic sampling over several weeks can help stage infection. An increase in titer or prevalence indicates active infection. Alternatively, sentinel animals can be added to seropositive colonies to detect active infection. Irrespective of serologic results, histopathology, immunohistochemistry (which can be performed on formalin-fixed, paraffin-embedded sections) and, where possible, virus isolation should be used to confirm infection. Virus can be isolated from the respiratory tract for up to 2 weeks, with peak titers occurring at about 9 days postinfection. Nasopharyngeal washings or lung tissue homogenates are most reliable and should be inoculated into embryonated hens' eggs or BHK-21 cell monolayer cultures. SV infection of cultured cells is noncytolytic, so erythrocyte agglutination or antigen detection methods must be used. RT-PCR also can be used to detect virus in infected lungs (Wan *et al.,* 1995).

Differential diagnosis. Respiratory infection caused by pneumonia virus of mice (PVM) is generally milder or asymptomatic. Histologically, necrosis of airway epithelium is less severe. Bacterial pneumonias of mice, including murine respiratory mycoplasmosis, are sporadic and can be differentiated morphologically and by isolation of causative organisms. Because SV pneumonia may predispose the lung to opportunistic bacterial infections, the presence of bacteria should not deter evaluation for a primary viral insult.

Control and prevention. Sendai virus infection is self-limiting in surviving immunocompetent mice. Suckling mice from immune dams are protected from infection by maternal antibody until after weaning. Control and eradication measures must eliminate exposure of susceptible animals, so that infection can "burn out." This is most easily accomplished by a quarantine period of 4–6 weeks wherein no new animals are introduced either as adults or through breeding. Control also is aided by the fact that Sendai virus is highly labile. Barrier housing is preferred for prevention and for control of transmission. Vaccination with Formalin-killed virus can provide short-term protection of valuable mice but is not commonly used for prevention.

Research complications. Sendai virus can cause immunosuppression and can inhibit growth of transplantable tumors. This effect has been attributed to virus-induced modification of tumor cell surface membranes. Pulmonary changes during SV pneumonia can compromise interpretation of experimentally induced lesions and may lead to opportunistic infections by other bacteria. They also have been associated with breeding difficulties in mice. This sign is thought to be an indirect effect due to stress, fever, or related changes during acute infection.

i. Pneumonia Virus of Mice (PVM) Infection (Parker and Richter, 1982)

Etiology. Pneumonia virus of mice (PVM) is an enveloped RNA virus in the genus *Pneumovirus* of the Paramyxoviridae. All isolates appear to have similar physicochemical, biological, and antigenic properties. The virus agglutinates erythrocytes of several rodent species, including mice. It replicates well *in vitro* in BHK-21 cells but, as with SV, is noncytolytic in cultured cells.

Clinical signs. Natural PVM infection in mice is asymptomatic. Therefore its name is clinically misleading, being derived from pneumonic illness that occurred after serial passage of the agent in mice. However, dyspnea, listlessness, and wasting may develop in immunodeficient mice infected with PVM (Weir *et al.*, 1988).

Epizootiology. PVM causes natural infections of mice, rats, hamsters, and probably other rodents and may be infectious for rabbits. Serological data indicate that PVM is prevalent in mice (Jacoby and Lindsey, 1997) and has a worldwide distribution. However, it appears to spread less rapidly than Sendai virus. Intimate contact between mice is probably required for effective transmission. This characteristic may reflect the fact that environmental inactivation of virus occurs rapidly. Infection appears to be acute and self-limiting in immunocompetent mice but may persist in immunodeficient mice.

Pathology. PVM replicates exclusively in the respiratory tract and reaches peak titers in the lung 6–8 days after infection. Although pulmonary consolidation can occur in experimentally infected mice, gross lesions are rare during natural infection. Histological lesions can occur in the upper and lower respiratory tract. They consist of mild necrotizing rhinitis, necrotizing bronchiolitis, and interstitial pneumonia, which usually occurs within 2 weeks after exposure to virus and is largely resolved by 3 weeks. The predominant inflammatory infiltrate is compromised of mononuclear cells, but some neutrophils are usually present. Immunohistochemistry on paraffin-embedded tissues can be used to detect viral antigen in bronchial epithelium, alveolar macrophages, and possibly alveolar epithelium during acute infection. Residual lesions include nonsuppurative perivasculitis, which can persist for several weeks after acute infection has ceased. Severe pneumonia can occur in immunodeficient mice, as noted above. It is characterized by generalized pulmonary consolidation that reflects severe interstitial pneumonia with inflammatory exudates and desquamated alveolar pneumocytes filling alveolar spaces (Fig. 25).

Diagnosis. Diagnosis is based primarily on serological detection that can be supplemented by histopathology, immunohistochemistry, *in situ* hybridization, and virus isolation. Reliable IFA and ELISA assays are available (London *et al.*, 1983). Virus replication in BHK-21 cells is detected by immunofluorescence or other antigen detection methods. Virus also can be detected in tissues by RT-PCR (S. R. Compton, personal communication, 2001).

Differential diagnosis. Because PVM is antigenically distinct from other murine viruses, serology is the most useful method to separate PVM infection from other respiratory infections of mice. However, in immunodeficient mice, where clinical signs

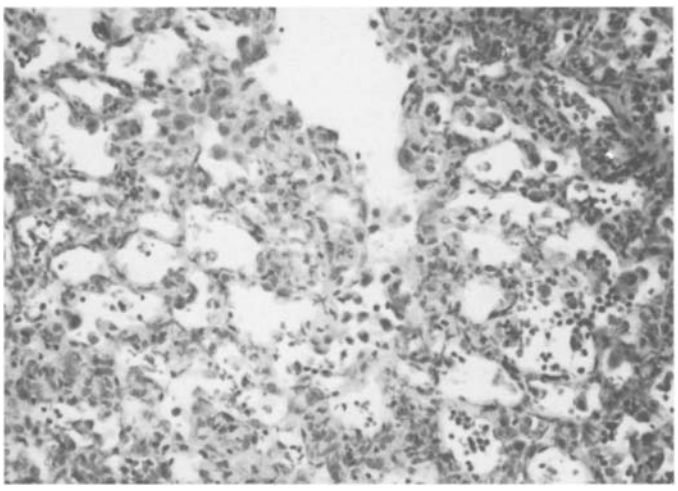

Fig. 25. Interstitial pneumonia caused by pneumonia virus of mice.

and lesions are typical, it must be differentiated from other pneumonias, especially those due to SV and *Pneumocystis carinii.* Additionally, PVM can coexist with and exacerbate *P. carinii* infection in immunodeficient mice (Bray *et al.,* 1993). Therefore, the careful application of immunohistochemistry and argyrophilic stains is recommended for definitive diagnosis of exudative interstitial pneumonia in mice.

Prevention and control. PVM infection is acute and self-limiting in immunocompetent mice. Seropositive mice should be viewed as either immune or in the final stages of acute infection. Therefore control and prevention follows guidelines applicable to SV infection.

Research complications. PVM can exacerbate pneumocystosis, as noted above.

j. Reovirus Infections

Two members of the family Reoviridae infect laboratory mice: reovirus per se and murine rotavirus, also known as epizootic diarrhea of infant mice (EDIM) virus.

i. Reovirus 1, 2, or 3 (Tyler and Fields, 1986)

Etiology. Reoviruses of mammals have been divided into three serotypes: reovirus 1, 2, and 3 . A number of wild-type and laboratory strains have been characterized, and related viruses have been recovered from mammals, marsupials, birds, insects, and reptiles. The virion contains segmented, double-stranded RNA and is relatively heat stable. Natural infections in mice are usually not caused by pure serotypes, because reoviruses actively recombine. Reoviruses replicate well in BHK-21 cells and other continuous cell lines, as well as in primary monolayer cultures from several mammals.

Clinical signs. Clinical disease is rare and age dependent. Acute disease affects sucklings at about 2 weeks of age, whereas adults usually have asymptomatic infection. Signs in sucklings include emaciation, abdominal distension, and oily, matted hair due to steatorrhea. Icterus may develop and is most easily discerned as discoloration in the feet, tail, and nose. Incoordination tremors and paralysis occur just before death. Convalescent mice are often partially alopecic and are typically runted. Alopecia, runting, and icterus may persist for several weeks, even though infectious virus can no longer be recovered. Infants born to immune dams are protected from disease by maternal immunity.

Epizootiology. The prevalence of reovirus 3 infection in colonies in the United States ranges from about 5 to 20% (Jacoby and Lindsey, 1997). Reoviruses are highly infectious among infant mice and can be transmitted by the oral–fecal or aerosol routes, but mechanical transmission by arthropods has also been documented. Additionally, virus may be carried by transplantable neoplasms and transmitted inadvertently by injection. Transmission is inefficient among adult mice. There is no evidence that vertical transmission is important or that genetic resistance or gender influence expression of disease. Infection in immunocompetent mice appears to be self-limiting, lasting up to several weeks but terminating with the development of host immunity. Nevertheless, the presence of a carrier state has not been excluded. The course of infection in immunodeficient mice should be considered prolonged, but the duration has not been determined.

Pathology. Reovirus 3 can cause severe pantropic infection in infant mice. After parenteral inoculation, virus can be recovered from the liver, brain, heart, pancreas, spleen, lymph nodes, and blood vessels. Following ingestion, reoviruses gain entry by infecting intestinal epithelial cells (M cells) that cover Peyer's patches. Virus can be carried to the liver in leukocytes, where it is taken up by Kupffer's cells prior to infecting hepatocytes.

In acute disease, livers may be large and dark, with yellow foci of necrosis. The intestine may be red and distended, and, in infants, intestinal contents may be bright yellow. Myocardial necrosis can evoke pale epicardial foci, and pulmonary hemorrhages have been reported. Myocardial edema and necrosis are especially prominent in papillary muscles of the left ventricle. The brain may be swollen and congested. Central nervous system lesions are most prevalent in the brain stem and cerebral hemispheres. Neuronal degeneration and necrosis are followed quickly by meningoencephalitis and satellitosis. Severe encephalitis may evoke focal hemorrhage. In the chronic phase, wasting, alopecia, icterus, and hepatosplenomegaly may persist. Orally infected suckling mice can develop multifocal hepatocytic necrosis, which may include the accumulation of dense eosinophilic structures resembling Councilman bodies. Hepatocytomegaly, Kupffer's cell hyperplasia, and intrasinusoidal infiltrates of mononuclear cells and neutrophilic leukocytes also can develop. In experimentally inoculated mice, necrotic foci can persist in the liver for at least 4 weeks. Chronic active hepatitis may develop after acute infection and result in biliary obstruction. Acinar cells of the pancreas and salivary glands can undergo degeneration and necrosis. Because pancreatic duct epithelium is susceptible to infection, parenchymal lesions in pancreas may be caused by obstruction rather than by viral invasion of parenchyma. Pulmonary hemorrhage and degeneration of skeletal muscles also have been observed. Both humoral and cellular immunity seem to participate in host defenses, but it is unclear how host immunity may influence the course of chronic infection.

Infection with reovirus 1 results in a similar distribution of significantly milder lesions. However, reovirus 2 is highly en-

terotropic, inducing mild enteritis without lesions in other tissues (Barthold *et al.,* 1993a).

Diagnosis. ELISA and IFA tests have been developed for the serological detection of reovirus 3 infection (London *et al.,* 1983), and viral RNA can be detected by RT-PCR (Steele *et al.,* 1995). A presumptive diagnosis of reovirus 3 infection is aided clinically by detection of the oily hair effect, accompanied by jaundice and wasting. The presence histologically of multisystemic necrosis is consistent with severe reovirus 3 infection but should be confirmed by immunohistochemistry or virus isolation.

Differential diagnosis. Reovirus 3 infection must be differentiated from other diarrheal diseases of infant mice, including those caused by mouse coronaviruses, EDIM virus, *Salmonella* spp., or *Clostridium piliforme.*

Prevention and control. Although surviving mice appear to recover completely from infection, the potential for a carrier state is unresolved. Therefore, it may be necessary, after adequate testing for the continued presence of virus by the use of sentinels, MAP testing, or other appropriate means, to rederive or replace infected stock. Prevention depends on adequate barrier husbandry coupled with adequate serological monitoring.

Research complications. Reovirus 3 infection can interfere with research in several ways. Infections in breeding colonies can result in high mortality among sucklings from nonimmune dams. Virus has been commonly recovered from transmissible neoplasms and is suspected of being oncolytic. The potential exists for interference with hepatic, pancreatic, cardiovascular, or neurological research.

ii. Rotavirus (EDIM virus) (Sheridan and Vonderfecht, 1986)
Etiology. Rotaviruses are double-stranded, segmented RNA viruses that have a wheel-like ultrastructural appearance. EDIM virus is a group A rotavirus that replicates in differentiated epithelial cells of the small intestine by budding into cisternae of endoplasmic reticulum. Currently, only a single antigenic strain is recognized, but antigenically distinct variants may exist. EDIM virus shares an inner capsid antigen with rotaviruses of rabbits, fowl, nonhuman primates, human beings, and domestic and companion animals. These agents tend to be species-specific under natural conditions and can be differentiated by serum neutralization tests. Cultivation of EDIM virus requires the presence of proteolytic enzymes to cleave an outer capsid polypeptide.

Clinical signs. Clinical signs occur in infant mice less than 2 weeks old. This age-related susceptibility also applies to infection in immunodeficient mice. Furthermore, clinical signs occur only in offspring of nonimmune dams, because maternal immunity protects infants until they have outgrown susceptibility to clinical disease (Rose *et al.,* 1998). The cardinal signs are diarrhea with fecal soiling of the perineum, which may extend to the entire pelage in severe cases. Despite high morbidity, mortality is low because affected mice continue to nurse. Transient weight loss does occur, and there may be a delay in reaching adult weight. Recovery from infection usually occurs in about 2 weeks and, once weight is regained, is clinically complete.

Epizootiology. EDIM virus appears to be infectious only for mice and occurs episodically in mouse colonies. However, infection is probably widespread geographically. Its prevalence in mouse colonies in the United States ranges between 5 and 25%, according to a recent survey (Jacoby and Lindsey, 1997). All ages and both sexes can be infected, but genetic resistance and susceptibility have not been determined. The virus is highly infectious and is transmitted by the oral–fecal route. Asymptomatically infected adult mice can shed virus in feces for at least 17 days, an interval that may be extended in immunodeficient mice (Riepenhoff-Talty *et al.,* 1995). After oral inoculation, virus is essentially restricted to the gastrointestinal tract, particularly the small and large intestine, although small amounts of virus may be present in liver, spleen, kidney, and blood. Nursing dams can contract infection from their litters. Transplacental transmission has not been demonstrated.

Pathology. Gross lesions occur primarily in the gastrointestinal tract, but thymic atrophy can result from infection-related stress. The intestine is often distended, flaccid, and filled with gray-green gaseous liquid or mucoid fecal material that soils the pelage. The stomach contains curdled milk, except in terminal cases with anal impaction. Virus preferentially infects terminally differentiated enterocytes in the small and large intestine, which accounts for the age-related susceptibility to disease; the number of such cells decreases as the intestinal tract matures. Characteristic histological lesions are most easily discerned in the small intestine in mice less than 2 weeks old (Little and Shadduck, 1987). They consist of increased vacuolation of villar epithelial cells with cytoplasmic swelling, which give villi a clubbed appearance (Fig. 26). The vacuoles must be differentiated from normal absorption vacuoles in nursing mice. The lamina propria may be edematous, but necrosis and inflammation are not prevalent.

Diagnosis. EDIM virus infection is detected serologically by IFA or ELISA (Ferner *et al.,* 1987). Clinical disease is diagnosed from signs and typical histological lesions in the intestine, which can be confirmed by immunohistochemical or ultrastructural demonstration of virus in intestine or in intestinal filtrates or smears. Rotavirus antigen can be detected in feces by

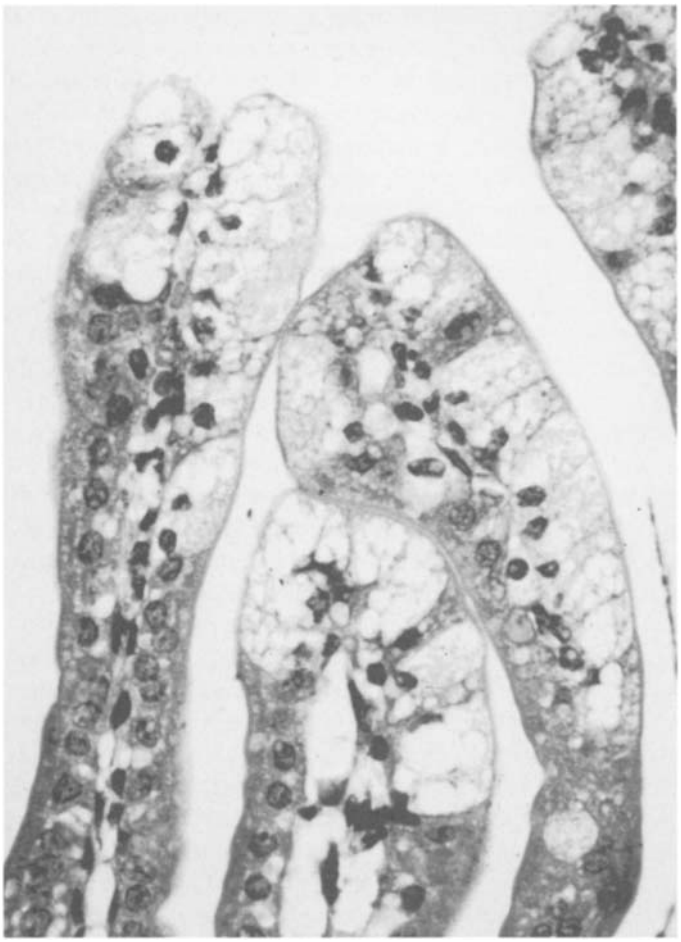

Fig. 26. Mouse rotavirus (EDIM) infection. Clubbing of intestinal villi accompanied by cytoplasmic swelling and vacuolization.

ELISA, but certain dietary ingredients can cause false-positive reactions. Infection can also be diagnosed by RT-PCR (Wilde *et al.,* 1990).

Differential diagnosis. EDIM virus infection must be differentiated from other diarrheal diseases of suckling mice such as intestinal coronavirus (mouse hepatitis) infection, reovirus 3 infection, Tyzzer's disease, and salmonellosis. The presence of milk in the stomach can be helpful in differentiating EDIM virus infection from more severe enteric infections, such as those caused by pathogenic coronaviruses, during which cessation of nursing often occurs. The possibility of dual infections must also be considered. Thymic necrosis in EDIM virus–infected mice, although nonspecific, must be differentiated from that due to mouse thymic virus (MTV) infection.

Prevention and control. The spread of EDIM can be controlled effectively by the use of microbarrier cages and good sanitation. Because infection appears to be acute and self-limiting, cessation of breeding for 4–6 weeks to allow immunity to build in adults while preventing access to susceptible neonates also is recommended. Alternatively, litters with diarrhea can be culled, in combination with the use of microbarrier cages. The duration of infection in immunodeficient mice has not been determined, but it is reasonable to assume that chronic infection occurs. Therefore, such animals should be eliminated. Litters from immune dams are more resistant to infection. Prevention of EDIM virus infection depends on maintenance of sanitary barrier housing with adequate serological surveillance.

Research complications. The research complications of EDIM infection pertain to clinical illness with diarrhea and retarded growth. Transient thymic necrosis may perturb immunological responses.

k. *Mouse Coronavirus (Mouse Hepatitis Virus [MHV]) Infection (Compton et al., 1993)*

Etiology. Mouse coronaviruses are large, pleomorphic, enveloped RNA viruses with radially arranged peplomers (spikes). Early clinical and laboratory investigations emphasized their potential to induce hepatitis, so their original designation, which is still used actively, is mouse hepatitis virus (MHV). Experience has shown, however, that hepatitis is not a common feature of natural infection in immunocompetent mice. Five prototype strains have received much attention from research scientists. They are: JHM (MHV4), MHV-1, MHV-3, MHV-S, and MHV-A59. However, numerous additional strains have since been identified that differ in virulence, tissue tropism, and antigenicity. This reflects the fact that mutation is common among coronaviruses, a property that increases risks for recurrent infection. As described further below, MHV isolates are often categorized according to their organotropism into two biotypes: *enterotropic* strains, which infect primarily the intestinal tract, and *polytropic* strains, which initially infect the respiratory tract but often progress to multisystemic dissemination (Homberger, 1997). However, isolates may contain features of both biotypes. Although MHV isolates and strains share internal antigens (M and N), they can be distinguished by neutralization tests that detect strain-specific spike (S) antigens. MHV shares antigens with the coronaviruses of rats, a finding that has been exploited to develop heterologous antigens for serological tests. MHV also is related to human coronavirus OC43.

A number of established cell lines can be used for propagating prototype MHV strains *in vitro.* However, field isolates are difficult to maintain *in vitro.* NCTC 1469 mouse liver cells are useful for growing many polytropic strains. Enterotropic strains have been grown in CMT-93 cells derived from a rectal carcinoma in a C57BL mouse but are generally difficult to propagate in cell culture. MHV can also be grown in mouse macrophages, cells that have been used for genetic studies of resistance and

Fig. 27. MHV syncytia in the intestine. Immunofluorescence stain.

susceptibility to infection. Irrespective of cellular substrate used for isolation or propagation, syncytium formation is emblematic of MHV infection. (See Fig. 27 for an example of syncytia *in situ.*)

Clinical signs. The prevalence and severity of clinical signs depend primarily on the age, strain, and immunological status of infected mouse and strain and tropism of virus (Barthold *et al.,* 1993b). As with many murine viruses, infection is often clinically silent among immunologically competent mature mice. Clinical morbidity is most often associated with suckling mice less than 2 weeks old or with immunodeficient mice. Suckling mice develop signs in various combinations that include diarrhea, inappetance, dehydration, weight loss, lassitude, and ruffled pelage, often terminating in death (Fig. 28). Neurotropic strains such as MHV-JHM may induce flaccid paralysis of the hindlimbs, but this sign is rarely encountered alone during natural infection. Conjunctivitis, convulsions, and circling may be seen occasionally. Mildly pathogenic strains may not cause acute disease in athymic mice but rather can cause a progressive wasting syndrome that may be accompanied by progressive paralysis.

Epizootiology. MHV infection is, for all practical purposes, an affliction of mice and arguably the most common viral infection in this species. A recent national survey reported MHV in nearly 60% of more than 100 major vivariums, usually among conventionally housed mice but also in more than 10% of barrier-housed colonies (Jacoby and Lindsey, 1997). There are no reports of natural transmission from mice to other species, but suckling rats can develop necrotizing rhinitis after intranasal inoculation with MHV-S. Sex-related or seasonal differences in susceptibility have not been found. MHV should be considered highly contagious, with natural transmission occurring by respiratory or oral routes. Recent reports suggest that enterotropic biotypes predominate in natural infections (Homberger *et al.,* 1998). Feces and nasopharyngeal exudates can serve as sources of infection. Natural vertical transmission has not been demonstrated. Introduction of MHV through injection of contaminated biologicals can be an important factor in epizootics, especially because some isolates infect B lymphocytes and, by implication, hybridomas nonlytically.

Infection in immunocompetent mice is self-limiting. Immune-mediated clearance of virus associated with seroconversion usually begins about a week after infection, and mice recover fully within 3–4 weeks. Humoral and cellular immunity appears to participate in host defenses to infection, and T cell–dependent immunity is an absolute requirement. Thus, age-related resistance to MHV correlates with maturation of lymphoreticular tissues. Enzootic infection had been construed to include persistent infection in individual mice. Current evidence suggests, however, that enzootic infection results either from the fresh and continuous introduction of immunologically naive or deficient mice or from the recurrent infection of immune mice with MHV variants that arise by natural mutation. Mutation is favored by immune pressures in enzootically infected colonies as well as missteps during natural replication, which include copying errors and recombination. Thus, mice that have developed immunity to one strain of MHV can remain susceptible to one or more genetically and antigenically divergent strains, resulting in reinfection (Barthold and Smith, 1989a,b; Homberger *et al.,* 1992). This caveat has practical importance for breeding colonies. Maternal immunity protects suckling mice against homologous MHV strains but not against antigenically variant strains. However, maternal immunity, even to homologous strains, depends on the presence of maternally acquired antibody in the lumen of the intestine. Therefore, the

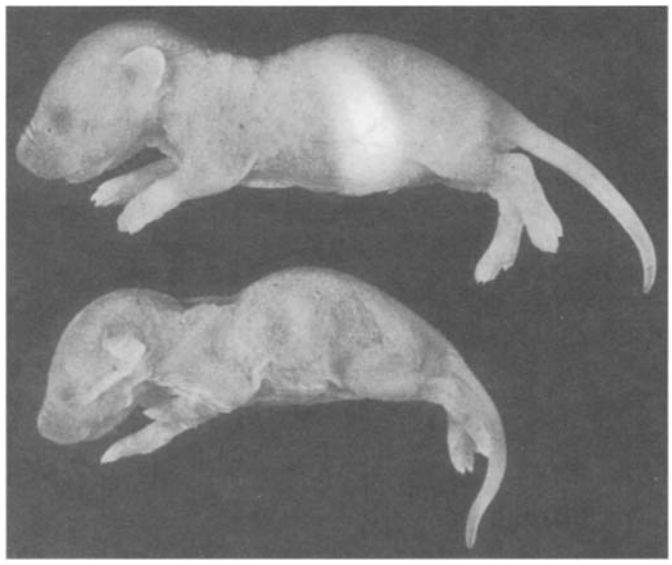

Fig. 28. Infant mice with enterotropic MHV infection. Upper mouse appears normal and has a milk-filled stomach. Lower mouse is runted and dehydrated and has an empty stomach. (From Barthold *et al.,* 1982.)

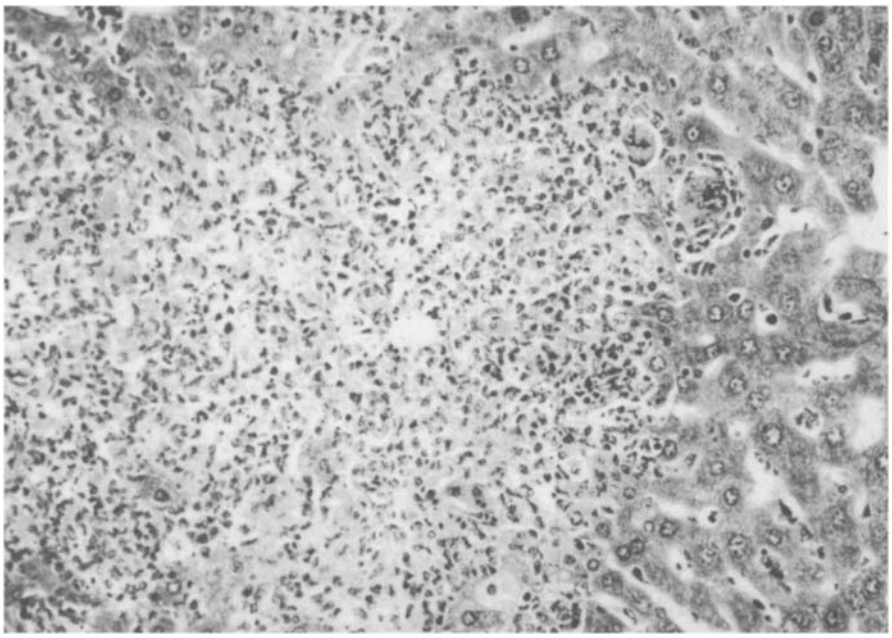

Fig. 29. Necrosis, inflammation, and syncytia in the liver of a mouse with MHV.

susceptibility of young mice increases significantly at weaning.

Strain differences in resistance and susceptibility can be inherited as an autosomal dominant trait (Barthold and Smith, 1987). For example, DBA/2 mice are highly susceptible to MHV-3 and die acutely even as adults, whereas A/J mice develop resistance to lethal infection shortly after weaning. However, genetic resistance also is virus strain–dependent. Therefore, mice resistant to one strain of MHV may be susceptible to another strain. It also is worth noting that the expanded use of genetically altered mice with novel or unanticipated deficits in antiviral responses may alter the outcome of virus–host interactions unpredictably. This pertains to MHV as well as other agents. For example, MHV infection has presented as granulomatous peritonitis and pleuritis in interferon-gamma knockout mice (France *et al.,* 1999).

Pathology. Polytropic strains replicate initially in the nasal mucosa, where necrotizing rhinitis may occur. Viremic dissemination can follow if virus gains access to regional blood vessels and lymphatics. Thus, viremia leads to secondary infection of vascular endothelium and parenchymal tissues in multiple organs including liver, brain, lymphoid organs, and other sites. Mice also may develop central nervous system disease by direct extension of infection from the olfactory mucosa along olfactory tracts. At necropsy, yellow-white foci indicative of necrosis can occur in multiple tissues, with the involvement of the liver as the classical lesion. Liver involvement may be accompanied by icterus and peritonitis. Histologically, necrosis can be focal or confluent and may be infiltrated by inflammatory cells (Fig. 29). Syncytia commonly form at the margin of necrotic areas and, in mild infections, may develop in the absence of frank necrosis. Syncytia formation is a hallmark of infection in many tissues, including intestine (Fig. 27), lung, liver, lymph nodes, spleen, thymus, brain, and bone marrow and in vascular endothelium in general. Although syncytia are transient in immunocompetent mice, they are a persistent feature in chronically infected, immunodeficient mice (Fig. 30). Neurotropic variants cause acute necrotizing encephalitis or meningoencephalitis in suckling mice, with demyelination in the brain stem and in peri-ependymal areas secondary to viral invasion of oligodendroglia. Convalescent mice may have residual mononuclear cell infiltrates around vessels or as focal lesions in the liver. Immunodeficient mice can develop smoldering necrotic lesions in the liver and elsewhere. Compensatory splenomegaly may occur because of expansion of hematopoietic tissue.

Enterotropic strains infect primarily the intestine and associated lymphoid tissues, although some may also cause systemic lesions, especially in liver and brain. The most common sites are terminal ileum, cecum, and proximal colon. The severity of disease is age-related, with immunocompetent young infants being at highest risk for lethal infection. Pathogenic strains can cause lesions ranging from villus attentuation and atrophy to fulminant necrotizing enterotyphlocolitis, which can kill suckling mice within several days (Fig. 31). The stomach is often empty, and the intestine is filled with watery to mucoid yellowish, sometimes gaseous contents. Hemorrhage or rupture of the intestine can occur. Syncytia are a consistent feature in viable mucosa and not only are formed in intestine but also may be pres-

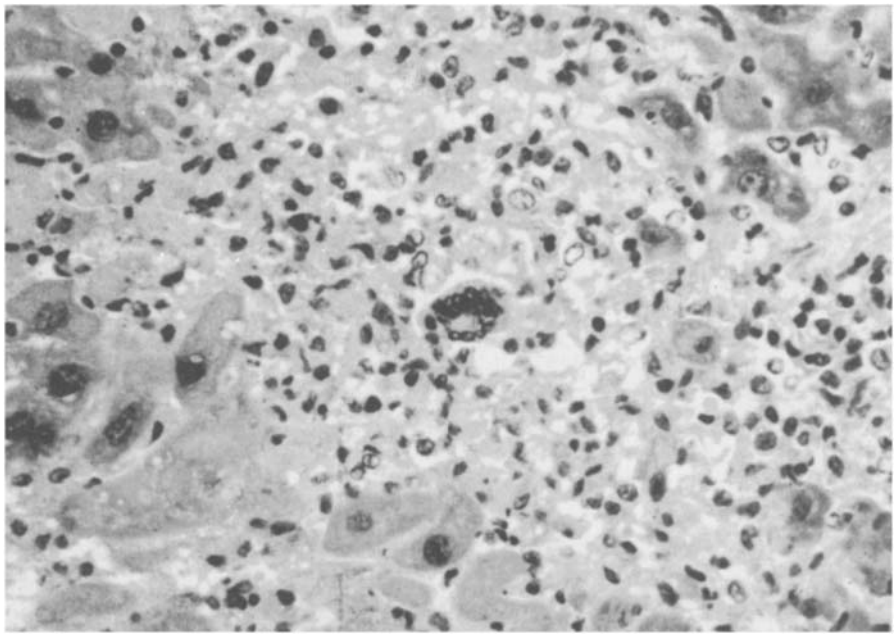

Fig. 30. Hepatitis and a syncytium (center) in the liver of an athymic mouse with MHV.

ent in mesenteric lymph nodes and endothelium of mesenteric vessels. Enterocytes may contain intracytoplasmic inclusions, but they are not diagnostic. Surviving mice develop compensatory mucosal hyperplasia, which eventually recedes. Older, more resistant mice usually develop transient syncytia without necrotic lesions. The exception occurs in immunodeficient mice, such as athymic and SCID mice, which can develop chronic proliferative bowel disease of varying severity and accompanied by syncytia (Fig. 32) (Barthold *et al.*, 1985).

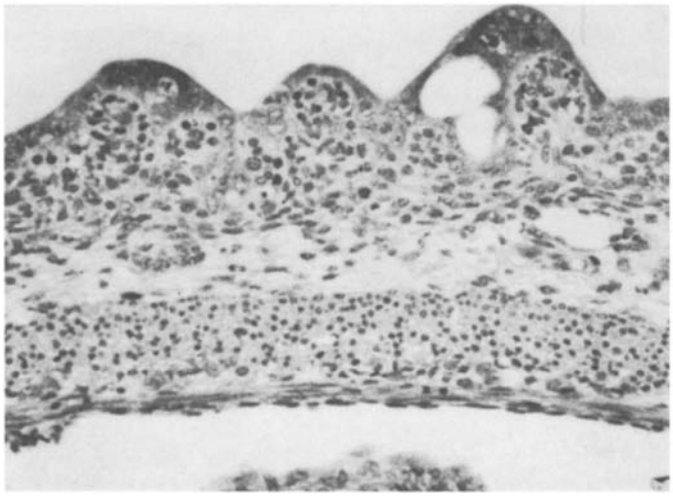

Fig. 31. Typhlocolitis with syncytia formation and effacement of mucosal architecture in an infant mouse with enterotropic MHV infection. (Courtesy of Dr. S. W. Barthold.)

Diagnosis. Because MHV infection is usually asymptomatic, serological testing is the most reliable diagnostic tool. Many animal resources rely on sentinel mouse protocols for continuous serological surveillance, because immune dysfunction can delay or abrogate seroconversion among research mice. ELISA and IFA tests are well established, sensitive, and reliable (Smith, 1983a; Smith and Winograd, 1986). Neutralization tests are used to differentiate individual virus strains in the research laboratory but are problematic for routine use, because of cost, technical complexity, and the effects of natural antigenic mutation inherent to MHV. Additionally, strain-specific, serologic identification per se does not predict biological behavior, including virulence or tissue tropism. Serology also can be used in the context of mouse antibody production assays in which adult mice are inoculated with suspect tissues to elicit seroconversion. Molecular diagnostics are being applied more widely for the diagnosis of MHV infection. PCR protocols to detect virus in tissues or excreta are available (Casebolt *et al.*, 1997; Homberger *et al.*, 1991; Yamada *et al.*, 1993), and access to testing can be obtained from specialized laboratories. Clinical signs of diarrhea, acute death, or neurological deficits are suggestive but not pathognomonic. The detection of syncytia augmented, when possible, by immunohistochemistry to detect MHV antigens (Fig. 33) is a useful and practical means to confirm infection (Brownstein and Barthold, 1982). This strategy should attempt to select mice that are in early stages of infection, because necrosis in infant mice or seroconversion in older mice may reduce the chances of detecting syncytia or viral antigens. The option of using immunodeficient mice as sentinels can be considered,

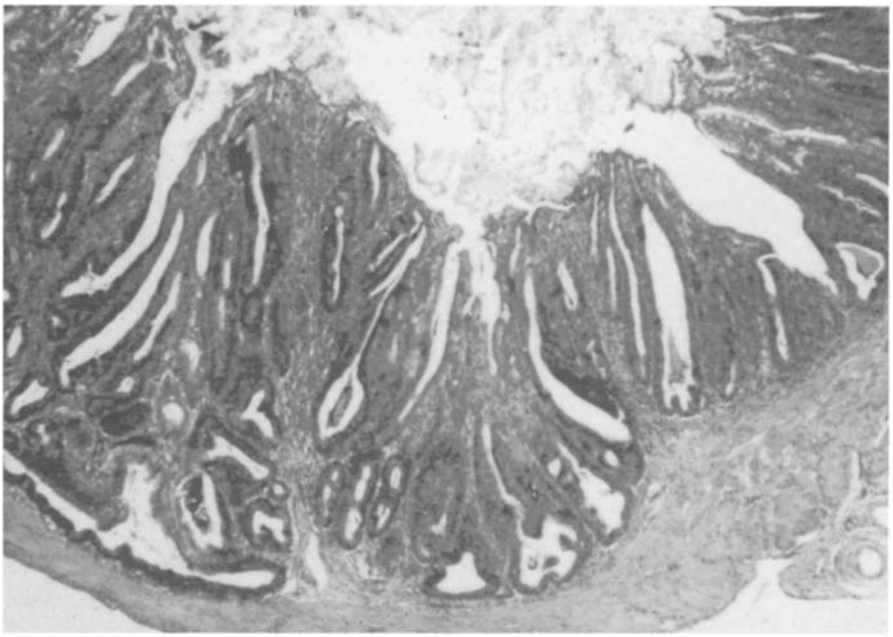

Fig. 32. Proliferative enteritis in an athymic mouse with MHV.

because they sustain prolonged infection. However, they should be securely confined because they also amplify virus loads. If properly controlled, amplification in immunodeficient mice can, however, facilitate subsequent virus isolation in tissue culture.

Differential diagnosis. MHV infection must be differentiated from other infectious diseases that cause diarrheal illness, runting, or death in suckling mice and wasting disease in immunodeficient mice. These include EDIM, mousepox, reovirus 3 infection, Tyzzer's disease, and salmonellosis. Neurological signs or demyelinating lesions must be differentiated from mouse encephalomyelitis virus infection or noninfectious CNS lesions, such as neoplasms, including polyoma-induced tumors in athymic mice.

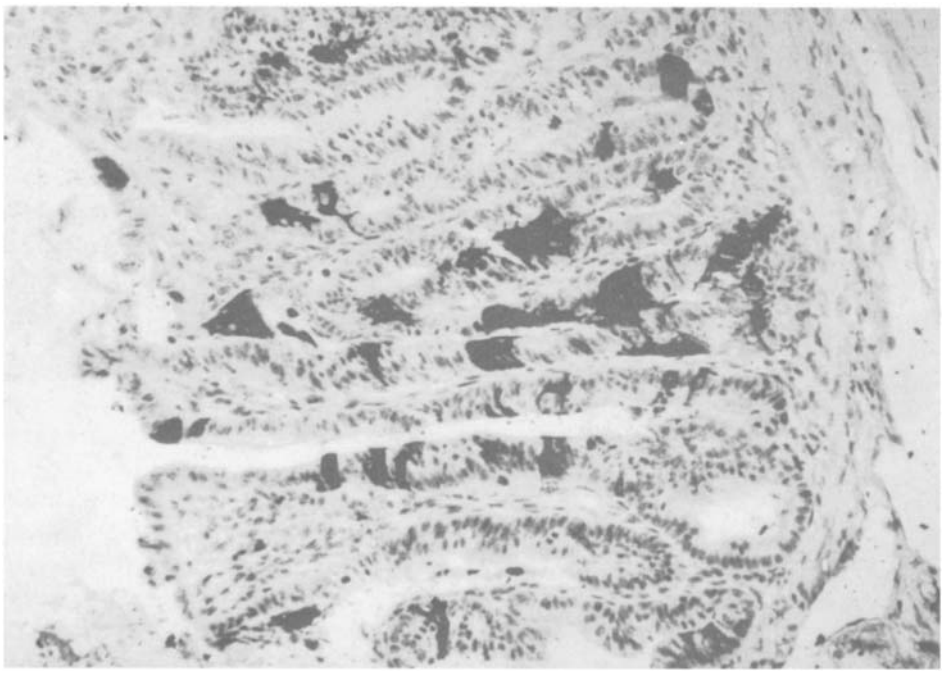

Fig. 33. MHV antigen in the small intestine, detected by enzyme immunohistochemistry.

Prevention and control. Control and prevention of MHV infection can be difficult because of the numerous variables that influence its expression. Perhaps the most important factor is the duration of infection in individual mice and in mouse colonies. There is evidence that infection in an individual immunocompetent mouse is acute and self-limiting. Such mice can be expected to develop immunity and eliminate virus within 30 days. Therefore, quarantine with the temporary cessation of breeding can be used effectively to eliminate infection (Weir *et al.,* 1987). Additionally, maternally derived immunity can protect infant mice from infection until they are weaned and moved to uncontaminated quarters (Homberger, 1992; Lipman *et al.,* 1987). Careful testing with sentinel mice should be used to assess the effectiveness of quarantine or "natural rederivation," as just described. This is especially true to account for the potential that a mutant variant could arise during quarantine and result in prolongation of colonywide infection. Immunodeficient mice, by contrast, are susceptible to chronic infection and viral excretion. Therefore, control measures must be more drastic and include depopulation. There also is an emerging gray zone, associated with the creation of genetically altered mice, in which decisions about control strategies are more problematic. It includes mice with unrecognized or unanticipated immune dysfunction or with selective immune dysfunction in which the impact of MHV infection is not known. Such colonies, which may contain highly valuable or irreplacable mice, may be rescued by cesarean rederivation or embryo transfer if vertical transmission of MHV infection is subsequently ruled out. Although rodent coronaviruses are not viable for extended periods in the environment, excreted virus may remain infectious for up to several days, so proper sanitation and disinfection of caging and animal quarters as well as stringent personal sanitation are essential to eliminate infection.

The prevention of MHV requires procurement of animals from virus-free sources and maintainence under effective barrier conditions monitored by a well-designed quality assurance program. Control of feral mouse populations, proper husbandry and sanitation, and strict monitoring of biological materials that may harbor virus (e.g., transplantable neoplasms, cell lines) are also important strategies to prevent adventitious infection.

Research complications. Numerous research complications have been attributed to MHV (Compton *et al.,* 1993; Homberger, 1997), and the unpredictable outcome of infection in genetically altered mice is likely to lengthen the list. For example, and apart from its clinical impact, MHV may stimulate or suppress immune responses, contaminate transplantable neoplasms, and be reactivated by treatment of asymptomatically infected animals with several classes of drugs, including immunosuppressive agents, and by intercurrent infections. It also can alter tissue enzyme levels. Additionally, the ubiquitous threat of MHV infection and uncertainty about its potential effects on a given research project provoke concerns that may exceed its true impact. For example, transient infection with a mild enterotropic strain is unlikely to disrupt systemic immune responses, whereas infection with a polytropic strain may be highly disruptive. This is not to say that asymptomatic or strictly enterotropic infection should be taken lightly but simply to caution against overreaction in assessing the impact of an outbreak.

l. Mouse Encephalomyelitis Virus (MEV) Infection (Downs, 1982; Lipton and Rozhon, 1986)

Etiology. MEV is a small, nonenveloped, RNA-containing cardiovirus. It was discovered by Max Theiler during experimental studies of yellow fever virus in mice, so it is also referred to as Theiler's MEV or simply TMEV. Established strains include TO (Theiler's original), FA, DA, and GD VII, the last of which is named after George Martine (George's disease), an assistant in Theiler's laboratory. The virus is rapidly destroyed by temperatures over 50°C and by alcohol but not by ether. It can be cultivated *in vitro* in several continuous cell lines, but BHK-21 cells are routinely used for isolation and propagation. It is antigenically related to encephalomyocarditis virus, which does not infect mice by natural exposure. As with other nonenveloped viruses, MEV is resistant to environmental inactivation, a factor that must be considered in control and prevention of infection.

Clinical signs. The development of clinical disease depends on virus strain, mouse strain, and route of exposure but is exceedingly rare (estimated at 0.1–0.01% of infected mice). When clinical signs occur, they are expressed as neurological disease. The characteristic sign is flaccid posterior paralysis, which may be preceded by weakness in the forelimbs or hindlimbs, but in mice which are otherwise alert (Fig. 34). Some mice may recover, but death frequently ensues, often because of failure to obtain food or water. Furthermore, mice that recover from the paralytic syndrome are disposed to a chronic demyelinating phase, which is expressed as a gait disturbance.

Epizootiology. Infection occurs primarily in laboratory mice with the exception of the MGH strain, which has been isolated from laboratory rats and is pathogenic in mice and rats after experimental inoculation. The prevalence of MEV in mouse colonies is low, a reflection of the slow rate at which virus is transmitted from mouse to mouse. MEV infection is acquired by ingestion and replicates primarily in the intestinal mucosa. Enteric infection can persist after the development of host immunity and can result in chronic or intermittent excretion of virus in feces over several months (Brownstein *et al.,* 1989). Mice often become infected shortly after weaning, but virus is seldom recovered in mice over 6 months of age. However, neurologic infection can persist—in the brain and spinal cord—for at least 1 year. In contrast to MHV, immunity to one strain

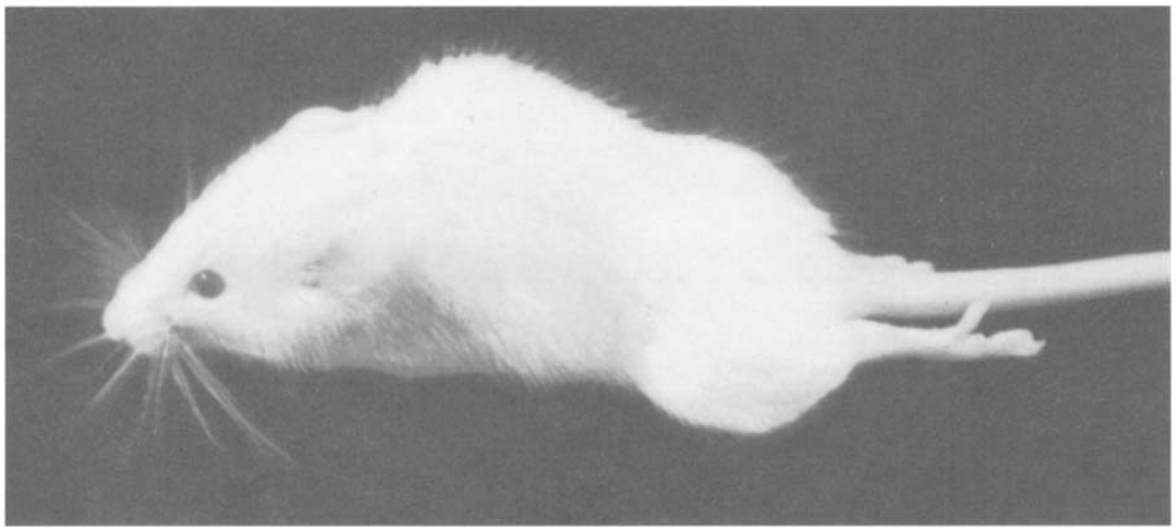

Fig. 34. Posterior paralysis in a mouse with MEV infection.

of MEV provides cross-protection to other strains. There are no reports of differences in mice with respect to susceptibility to infection under natural conditions. Prenatal transmission has not been found.

Pathology. Intestinal MEV infection does not cause lesions, but virus can be detected in enterocytes by immunohistochemistry or *in situ* hybridization. Poliomyelitis-like disease, the syndrome that may be encountered during natural infections, is characterized by acute necrosis of ganglion cells, neurophagia, and perivascular inflammation, which occurs particularly in the ventral horn of the spinal cord gray matter but also can involve higher centers such as the hippocampus, thalamus, and brain stem. During the subsequent demyelinating phase, mononuclear cell inflammation develops in the leptomeninges and white matter of the spinal cord, accompanied by patchy demyelination. The white-matter lesions are due to immune injury and are similar to those seen in experimental allergic encephalomyelitis (Monteyne *et al.*, 1997). Spontaneous demyelinating myelopathy, affecting the thoracic spinal cord and associated with MEV infection, has also been reported in aged mice. Virulent strains may cause acute encephalitis after experimental inoculation, whereas less virulent isolates produce acute poliomyelitis followed by chronic demyelinating disease.

Diagnosis. Infection is usually detected serologically by IFA or ELISA (Kraft and Meyer, 1986). A PCR assay also is available (Zoll *et al.*, 1993). Clinical signs are striking, if they occur, but are too rare to rely on for routine diagnosis. Histological lesions in the CNS and especially the spinal cord are characteristic when present. Virus can be isolated by inoculation of BHK-21 cells with intestine or CNS tissue.

Differential diagnosis. Neurotropic variants of MHV may, on occasion, cause similar neurological signs. Injury or neoplasia affecting the spinal cord can also produce posterior paralysis. Polyomavirus infection in athymic mice can induce tumors or demyelination in the CNS, which may result in clinical signs resembling those of MEV infection.

Prevention and control. Disease-free stocks were originally developed by foster-nursing infant mice. This technique or cesarean or embryo derivation techniques can be used successfully to eliminate infection (Lipman *et al.*, 1987). In either case, foster mothers should be surveyed in advance to ensure their MEV-free status. Selective culling can be considered as an option to eliminate infection, because infection spreads slowly. However, the virus is hardy in the environment and resists chemical inactivation, so it may be prudent to depopulate and disinfect rooms if the presence of infection is unacceptable.

Research complications. The principal hazard from MEV for research relates to its potential effects on the CNS.

2. Bacterial Diseases

a. Mycoplasmosis (Cassell et al., 1986; Lindsey et al., 1982, 1991i)

Several species of *Mycoplasma* can infect laboratory mice: *M. pulmonis, M. arthriditis, M. neurolyticum*, and *M. collis*. Antigenic cross-reactivity among these species, and especially between *M. pulmonis* and *M. arthriditis*, mandates that reliable diagnostic strategies incremental to serology be employed to distinguish potentially pathogenic infections. The following section first describes infection due to *M. pulmonis*, then summarizes infections associated with other murine mycoplasmas.

i. Mycoplasma pulmonis

Etiology. My. pulmonis is a pleomorphic, gram-negative

bacterium that lacks a cell wall and has a single outer limiting membrane. It causes murine respiratory mycoplasmosis (MRM).

Clinical signs. Mice are relatively resistant to florid MRM, so asymptomatic infection is common. When clinical signs occur, they reflect suppurative rhinitis, otitis media, and chronic pneumonia. Affected mice may display inactivity, weight loss, and ruffled hair coat, but the most prominent signs are "chattering" and dypsnea, due to rhinitis and purulent exudate in nasal passages. Otitis media may cause a head tilt, whereas suppurative inflammation in the brain and spinal cord, although rare, can cause flaccid paralysis. Naturally occurring disease of sites other than the respiratory tract has not been reported, but experimental infection of the genital tract can cause oophoritis, salpingitis, and metritis, which may lead to infertility or fetal deaths. Experimental inoculation of SCID mice has caused systemic infection accompanied by severe arthritis (Evengard *et al.,* 1994).

Epizootiology. MRM used to be a common infectious disease of mice, but improved housing, husbandry, and health care have reduced its prevalence dramatically. Although a recent survey suggests that *Mycoplasma* infection still affects about 15% of conventionally housed mouse colonies (Jacoby and Lindsey, 1997), the data did not differentiate *M. pulmonis* infections from those caused by less virulent species such as *M. arthritidis. Mycoplasma pulmonis* infection is contracted by inhalation and can occur in suckling and adult mice. Therefore, infection should be considered highly contagious. *In utero* infection has been demonstrated in rats but not in mice. Concomitant viral pneumonia (Sendai virus, mouse coronavirus) or elevated environmental ammonia concentrations may increase susceptibility to MRM. *Mycoplasma pulmonis* also infects rats, hamsters, guinea pigs, and rabbits. Among these species, only rats are significant reservoirs of infection for mice.

Pathology. Mycoplasma pulmonis is an extracellular organism that colonizes the apical cell membranes of respiratory epithelium. Attachment occurs anywhere from the anterior nasal passages to the alveoli and may be mediated by surface glycoproteins. The organism may injure host cells through competition for metabolites such as carbohydrates and nucleic acids or by release of toxic substances such as peroxides. Ciliostasis, reduction in the number of cilia, and ultrastructural changes leading to cell death have also been described. Detrimental effects on ciliated epithelium can lead to disrupted mucociliary transport, which exacerbates pulmonary disease.

Experimental expression of MRM is dose dependent. Doses of 10^4 colony-forming units (CFU) or less cause mild, transient disease involving the upper respiratory tract and middle ears, whereas higher doses often lead to acute, lethal pneumonia. Additionally, *Mycoplasma* strains can differ in virulence. Survivors of severe infection may develop chronic bronchopneumonia with bronchiectasis and spread infection to other mice. Intravenous inoculation of *M. pulmonis* can cause arthritis in mice, but arthritis is not a significant feature of natural infection.

Host genotype also is a major factor in the outcome of infection, with resistance being expressed phenotypically through the bactericidal efficiency of alveolar macrophages. Strains derived from a C57BL background appear to be resistant to pathogenic infection, whereas BALB/c, C3H, DBA/2, SWR, AKR, CBA, SJL, and others have varying degrees of increased susceptibility (Cartner *et al.,* 1996; Lai *et al.,* 1993).

The initial lesion of MRM is suppurative rhinitis, which may involve the trachea and major airways. Early inflammatory lesions, if not quickly resolved, progress to prominent squamous metaplasia. Transient hyperplasia of submucosal glands may occur, and lymphoid infiltration of the submucosa can persist for weeks. Syncytia can sometimes be found in nasal passages, in association with purulent exudate. Affected mice also develop suppurative otitis media and chronic laryngotracheitis with mucosal hyperplasia and lymphoid cell infiltrates. Pulmonary lesions are typified by bronchopneumonia, which spreads from the hilus. Lymphoid cells and plasma cells accumulate around bronchi which often contain neutrophils in their lumena. Chronic lung disease features suppurative bronchitis, bronchiolitis, and alveolitis (Fig. 35). Chronicity also increases the prevalence of bronchiectasis and abscessation.

Diagnosis. Accurate diagnosis should exploit the complementary use of clinical, serological, microbiological, molecular, and morphological methods. Clinical signs are variable but can be characteristic when they occur. A sensitive ELISA, a radioimmunosorbent assay, and a solid-phase radioimmunoassay are available for serological detection of infection but do not differentiate *M. pulmonis* infection from *M. arthritidis* infection (Cassell *et al.,* 1981). Further, some mice may be poor responders and develop very low antibody titers. Therefore attempts should be made to use other methods to confirm diagnosis. Primary among these are attempts to isolate the causative organism. The upper respiratory tract should be cultured because it is a common site for natural infection. Buffered saline or *Mycoplasma* broth should be used to lavage the trachea, larynx, pharynx, and nasal passages. Culture from the genital tract is warranted if this site is suspected. *Mycoplasma* species may be difficult to grow, so it is prudent to confirm that the relevant expertise and quality control exist in the diagnostic laboratory. Speciation can be accomplished by immunofluorescence or immunoperoxidase staining or by growth inhibition. Immunohistochemistry should be considered to supplement basic histopathologic examination. Immunofluorescence and immunoperoxidase techniques are available to identify mycoplasmal antigens in tissue sections or in cytological preparations of tracheobronchial or genital tract lavages (Brunnert *et al.,* 1994). More recently, PCR has been assessed as a diagnostic strategy

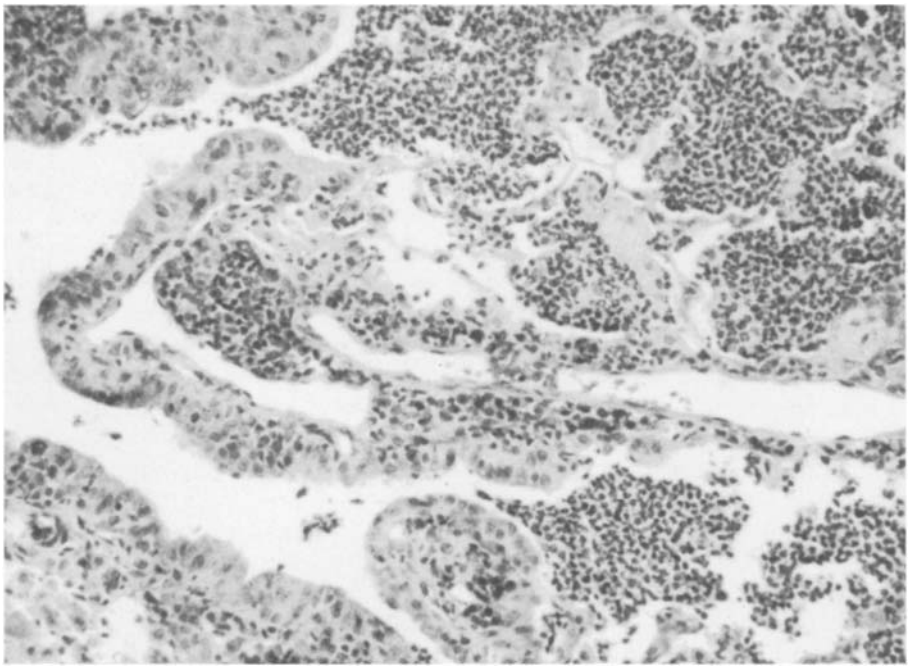

Fig. 35. Suppurative bronchopneumonia caused by *Mycoplasma pulmonis.*

(Brunnert *et al.,* 1994; Goto *et al.,* 1994; Harasawa *et al.,* 1990; Kunita *et al.,* 1990; Schoeb *et al.,* 1997; van Kuppeveld *et al.,* 1993). It appears capable of detecting infection rapidly, accurately, and sensitively in living mice or in paraffin-embedded tissues.

Differential diagnosis. MRM must be differentiated from bronchopneumonia associated with cilia-associated respiratory (CAR) bacillus. Silver stains may reveal CAR bacilli adherent to the respiratory epithelium. Sendai virus also can cause bronchopneumonia in mice but can be detected by serology and immunohistochemistry. Other causes of respiratory infection include pneumonia virus of mice, corynebacteriosis, and, in immunodeficient mice, *Pneumocystis carinii* infection. Combined infections with known pathogens or secondary opportunists also must be considered.

Prevention and control. Mice mount an effective immune response to *Mycoplasma pulmonis,* as measured by their recovery from mild infection and their resistance to infection after active or passive immunization (Cartner *et al.,* 1998). Antibodies of various classes are produced locally and systemically, but their role in infection is unclear. There is some evidence that antibody may facilitate phagocytosis of *M. pulmonis.* Classic cellular immunity, however, does not appear to play a major role in *M. pulmonis* infection in mice, because immunity cannot be transferred with immune cells. In addition, athymic and neonatally thymectomized mice are not more susceptible than imunocompetent mice to *M. pulmonis* pneumonia.

Host immunity aside, effective control and prevention of MRM depend primarily on maintenance of *Mycoplasma*-free colonies under barrier conditions supported by careful surveillance for infection by serology, microbiology, and histopathology. Cesarean or embryo rederivation can eliminate infection, but embryos, fetal membranes, and offspring must be tested to rule out contamination (Hill and Stalley, 1991). Treatment with tetracyclines suppresses clinical disease but does not eliminate infection. Some progress has been made in developing DNA-based vaccines against *M. pulmonis,* but they have not achieved clinical application (Lai *et al.,* 1997).

Research complications. Mycoplasma pulmonis can interfere with research by causing clinical disease or death. Experiments involving the respiratory tract, such as inhalation toxicology, can be compromised by chronic progressive infection. Additionally, affected mice are at greater risk during general anesthesia. *Mycoplasma pulmonis* may alter immunological responsiveness. For example, it is mitogenic for T and B lymphocytes and can increase natural killer cell activity. Perhaps one of the most important complications of *Mycoplasma* infection is contamination of cell lines and transplantable tumors.

ii. Other murine mycoplasmas *Mycoplasma arthritidis* is antigenically related to *M. pulmonis.* Therefore, serological evidence of mycoplasma infection must be supplemented by other diagnostic tests, as outlined above, to differentiate between these agents. Differentiation is important because *M. arthritidis,* though arthritogenic in mice after intravenous inoculation, is nonpathogenic during natural infection. *Mycoplasma collis*

has been isolated from the genital tract of the mouse but does not appear to cause natural disease.

Mycoplasma neurolyticum is the etiological agent of *rolling disease,* a rare syndrome which occurs within hours after intravenous inoculation of *M. neurolytica* exotoxin. Characteristic clinical signs include spasmodic hyperextension of the head and the raising of one foreleg followed by intermittent rolling on the long axis of the body. The rolling becomes more constant, but mice occasionally leap or move rapidly. After 1–2 hr of rolling, animals become comatose and usually die within 4 hr. All published reports of rolling disease are associated with experimental inoculation of organisms or exotoxin. Large numbers of organisms are needed to produce disease, and there is no indication that, under natural conditions, organisms replicate in the brain to concentrations required for the induction of these signs. Because animals are frequently inoculated with biological materials by parenteral routes, contamination with *M. neurolytica* may induce rolling disease inadvertently. Diagnosis can be made from the appearance of typical clinical signs, astrocytic swelling, and isolation of the causative organism. Clinical signs must be differentiated from rolling associated with *Pseudomonas*-caused otitis. *Mycoplasma pulmonis* has been recovered from the brain of mice but does not seem to cause overt neurological disease.

b. *Cilia-Associated Respiratory (CAR) Bacillus Infection*

CAR bacillus is a slender, gram-negative bacillus, which, in rats, produces clinical disease and lesions that closely resemble those of MRM (see Chapter 4). Chronic respiratory disease has been produced in mice by experimental inoculation, but natural clinical disease is rare (Griffith *et al.,* 1988). Furthermore, putative natural cases were reported in mice that were seropositive for Sendai virus and pneumonia virus of mice. Therefore, CAR bacillus may have acted as an opportunist rather than as a primary pathogen. On balance, one can assume that mice may contract natural infection, but attributing chronic respiratory disease in mice solely to CAR bacillus is not currently warranted.

An ELISA for serological detection of infection is available (Shoji *et al.,* 1988), and PCR-based diagnostics also have been developed (Goto *et al.,* 1995). Histologic assessment of infection requires the use of Warthin–Starry or similar stains to visualize argyrophilic bacilli adherent to the apical membranes of bronchial respiratory epithelium (Fig. 36). Alternatively, immunoperoxidase staining has also been used successfully to detect infection. There is a report that sulfamerazine (500 mg/liter) in drinking water may be effective in eradicating infection (Matsushita and Suzuki, 1995), but this strategy has not been confirmed. Alternative approaches for eradication are similar to those described for *Mycoplasma pulmonis.*

c. *Tyzzer's Disease (Fujiwara and Ganaway, 1994; Ganaway et al., 1971; Ganaway, 1982)*

Etiology. Tyzzer's disease is named for Ernest Tyzzer, who first described it in a colony of Japanese Waltzing mice. The causative organism, *Clostridium piliforme* (formerly *Bacillus piliformis*), is a long, thin, gram-negative spore-forming bacterium that appears to require living cells for *in vitro* growth. It

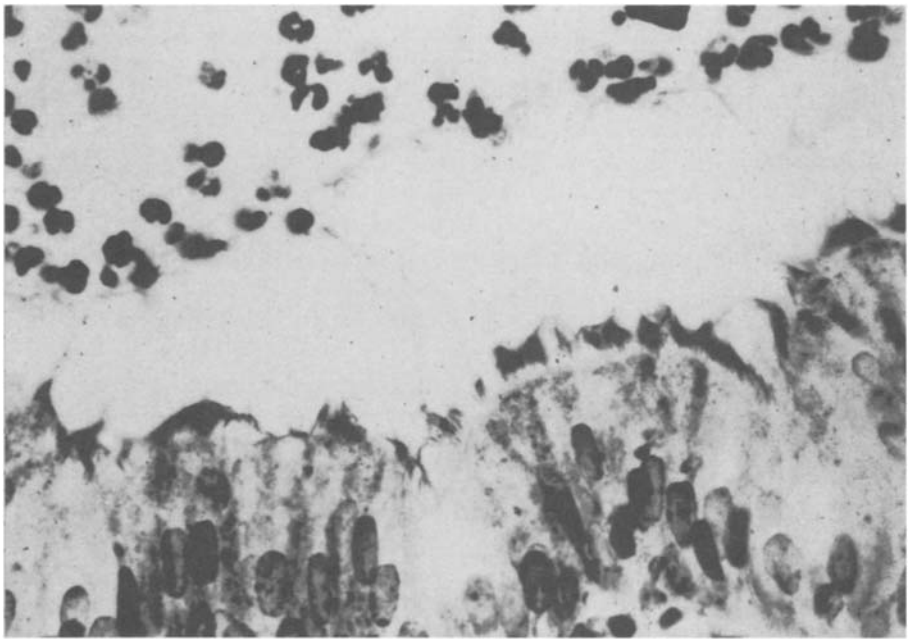

Fig. 36. CAR bacilli at the ciliated border of respiratory epithelium (Warthin–Starry stain). The adjacent bronchial lumen contains inflammatory cells.

has not been grown successfully on cell-free media, but it can be propagated by inoculation of susceptible vertebrates, the yolk sac of embryonated eggs, or hepatocyte cultures from mice (Ganaway *et al.,* 1985; Kawamura *et al.,* 1983).

Clinical signs. Clinical disease occurs as unexpected deaths that may be preceded by diarrhea and inactivity. Although outbreaks can be explosive and mortality is usually high, morbidity may be high or low. Additionally, subclinical infections can occur, accompanied by the development of antibodies to *C. piliforme.* Stresses, such as overcrowding, high temperature and humidity, moist food, and immunosuppression, may predispose mice to Tyzzer's disease. Susceptibility and resistance also are influenced by host genotype. It has been shown, for example, that C57BL/6 mice are more resistant than DBA/2 mice to Tyzzer's disease (Waggie *et al.,* 1981). Resistance to severe infection appears to be due, in part, to B lymphocyte function. Athymic mice also appear to have increased susceptibility to Tyzzer's disease (Livingston *et al.,* 1996). The role of T cells in resistance is not clear, because susceptibility among athymic mice appears to vary. However, the involvement of T cells can be inferred by the fact that several interleukins modulate resistance and susceptibility. Depletion of neutrophils or natural killer cells also increases susceptibility to infection.

Epizootiology. Current prevalence rates, reservoirs of infection, carrier states, and the mechanism of spread remain speculative. Tyzzer's disease occurs in many species of laboratory animals and in domestic and free-living species, but the reservoir of infection is unknown. Some strains appear capable of cross-infecting mice, rats, and hamsters, whereas others have a more restricted host range (Franklin *et al.,* 1994). Therefore the risks for cross-infection depend on the strain causing a given outbreak. The vegetative form of *C. piliforme* is unstable, but spores can retain infectivity at room temperature for at least 1 year and should be viewed as the primary means of spread. Natural infection is probably due to ingestion of organisms, which are subsequently shed in feces. Feces-contaminated food and soiled bedding are therefore the most likely sources of environmental contamination. Prenatal infection can be induced by intravenous inoculation of pregnant mice, but its importance in the natural transmission of infection has not been determined.

Pathology. Infection begins in the gastrointestinal tract, followed by bacteremic spread to the liver and, to a smaller extent, the heart. The lesions are characterized by necrosis in these tissues and in the mesenteric lymph nodes. Grossly, segments of the ileum, cecum, and colon may be red and dilated, with watery, fetid contents, whereas the liver, mesenteric lymph nodes, and heart often contain gray-white foci. Histologically, intestinal lesions include necrosis of mucosal epithelium, which may be accompanied by acute inflammation and hemorrhage. In the liver, foci of coagulation necrosis are generally distributed along branches of the portal vein, a finding compatible with embolic infection from the intestine. Peracute lesions are largely free of inflammation, but neutrophils and lymphocytes may infiltrate less fulminant lesions. Myocardial necrosis is sporadic in natural infection.

Bundles of long, slender rods occur in the cytoplasm of viable cells bordering necrotic foci, especially in the liver (Fig. 37) and intestine. They are found more easily during early stages of infection. Organisms in tissue sections do not stain well with hematoxylin–eosin stain. Silver stains (Warthin–Starry), Giemsa stains, or periodic acid–Schiff stains are usually required.

Diagnosis. Tyzzer's disease is diagnosed most directly by the demonstration of characteristic intracellular organisms in tissue sections of liver and intestine. Supplemental procedures include inoculation of cortisonized mice or embryonated eggs with suspect material, followed by histological or immunocytochemical demonstration of organisms in tissues. Asymptomatic infection can be detected by ELISA (Waggie *et al.,* 1987) or by PCR (Goto and Itoh, 1996).

Differential diagnosis. The histological detection of organisms is essential for differentiating Tyzzer's disease from other infections that can produce similar signs and lesions, especially mousepox, coronaviral hepatitis, reoviral hepatitis, helicobacteriosis, and salmonellosis. It also is important not to misconstrue extracellular rods as *Clostridium piliforme.*

Prevention and control. Barrier housing and husbandry that incorporate sanitation measures to avoid the introduction or buildup of spores in the environment are the bases for control or prevention of Tyzzer's disease. If infection occurs, spore formation will make control or elimination by antibiotic therapy problematic. Therefore, strict quarantine, followed by replacement of affected or exposed stock, must be considered. Rederivation by embryo transfer or cesarean section should take the potential for prenatal transmission of infection into account in housing and testing offspring. Thorough decontamination of the environment with an oxidizing disinfectant must be included in any control program. Additionally, procurement of food and bedding from suppliers with thorough quality assurance and vermin control programs is essential for both prevention and control. Husbandry supplies should be stored in vermin-proof quarters, and the option of heat sterilization of food and bedding should be considered.

Research complications. Research complications stem from clinical morbidity and mortality. Mice with immune dysfunction are at increased risk. There is recent evidence that infection causes elevations in selected cytokines (Van Andel, *et al.*, 2000).

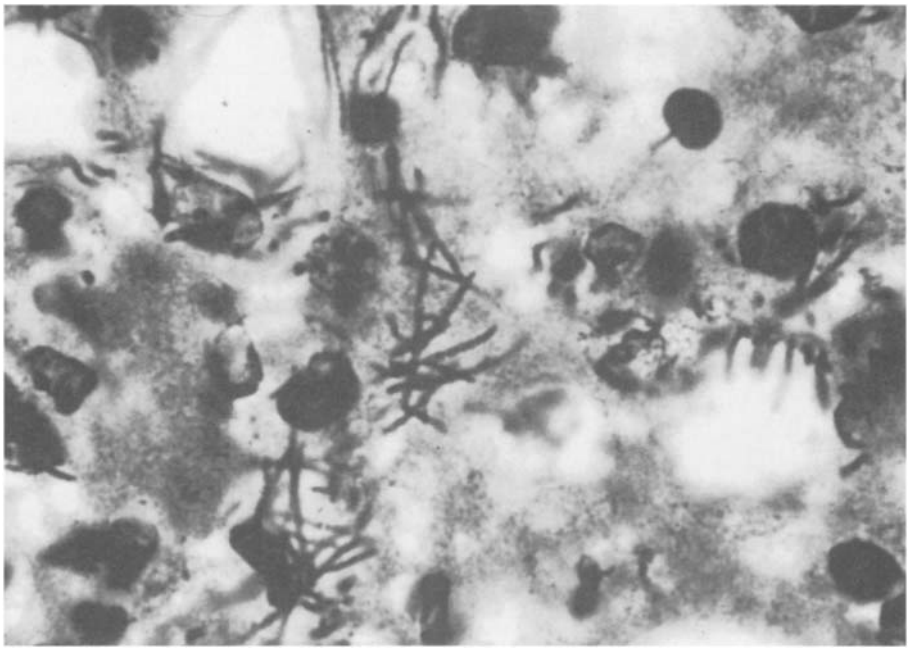

Fig. 37. *Clostridium piliforme* in the liver on a mouse with Tyzzer's disease (Warthin–Starry stain).

d. Transmissible Murine Colonic Hyperplasia

Etiology. The causative agent of transmissible murine colonic hyperplasia, *Citrobacter rodentium* (formerly *Citrobacter freundii* strain 4280), is a nonmotile, gram-negative rod that ferments lactose but does not utilize citrate or does so marginally (Barthold, 1980; Schauer *et al.,* 1995).

Clinical signs. Clinically apparent infection is characterized by retarded growth, ruffled fur, soft feces or diarrhea, rectal prolapse, and moderate mortality in older suckling or recently weaned mice (Barthold *et al.,* 1978).

Epizootiology. *Citrobacter rodentium* is not in the gastrointestinal flora of normal mice. It is thought to be introduced by contaminated mice, food, or bedding, from which it spreads by contact or additional fecal contamination. Host genotype can influence the course and severity of disease (Barthold *et al.,* 1977). For example, DBA, NIH Swiss, and C57BL mice are relatively resistant to mortality, whereas C3H/HeJ mice are relatively susceptible both as sucklings and as adults. Diet also can modulate infection, but specific dietary factors responsible for this effect have not been identified.

Pathology. *Citrobacter rodentium* attaches to the mucosa of the descending colon and displaces the normal flora. Attachment is accompanied by effacement of the microvillus border and formation of pedestal-like structures (attaching and effacing lesions) (Schauer *et al.,* 1993; Newman *et al.,* 1999). Colonization results in prominent mucosal hyperplasia, by unknown mechanisms. The characteristic gross finding is severe thickening of the descending colon, which may extend to the transverse colon and lasts for 2–3 weeks in surviving animals (Fig. 38) (Percy and Barthold, 2001a). Affected segments are rigid and either are empty or contain semiformed feces. Histologically, accelerated mitotic activity results in a markedly hyperplastic mucosa, which may be associated with secondary inflammation and ulceration (Fig. 39). Lesions subside after several weeks. Repair is rapid and complete in adults but slower in sucklings.

Diagnosis. Diagnosis depends on clinical signs, characteristic gross and histological lesions, and isolation of *C. rodentium* from the gastrointestinal tract or feces. The organism is relatively easy to culture on MacConkey's agar during early phases of infection, whereas the intestine may be free of aerobic bacteria during later stages. *Citrobacter rodentium* also can be detected by molecular hybridization (Schauer *et al.,* 1995).

Differential diagnosis. Transmissible murine colonic hyperplasia must be differentiated from other diarrheal diseases of mice, including infections caused by coronavirus, rotavirus, adenovirus, reovirus, *Salmonella, Clostridium piliforme,* and *Helicobacter* spp.

Prevention and control. Some success in curtailing epizootics has been achieved by adding antimicrobials to the drinking water (Barthold, 1980; Silverman *et al.,* 1979). Because *C.*

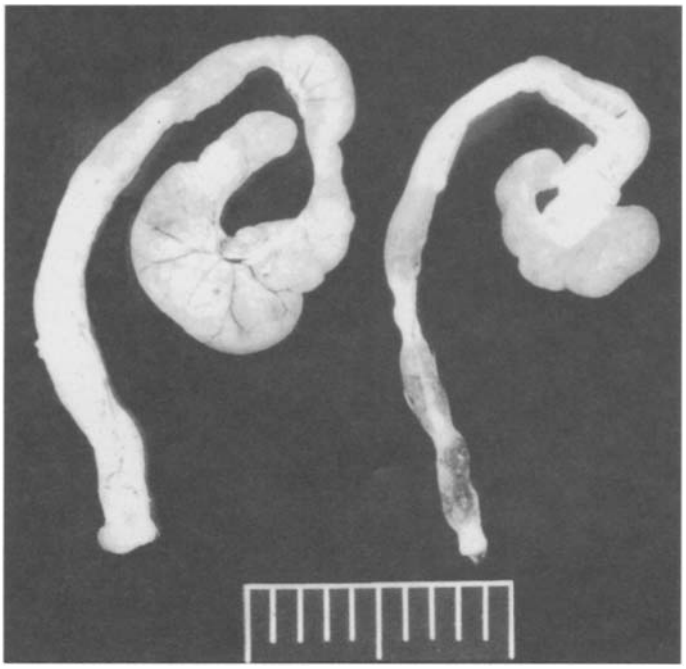

Fig. 38. Colons of a normal mouse (right) and of a mouse with transmissible murine colonic hyperplasia (left). The descending colon is thickened and opaque because of mucosal hyperplasia. (From Barthold *et al.*, 1978.)

rodentium may contaminate food, bedding, or water, proper disinfection of such materials is prudent before they are used for susceptible animals. Additionally, quarantine or the employment of microbarrier caging can reduce transmission. Surveillance for *C. rodentium* should be incorporated into quality assurance programs.

Research complications. The potential effects on research of colonic hyperplasia as a clinically severe disease are obvious. Colonic hyperplasia has been shown to increase the sensitivity of colonic mucosa to chemical carcinogens and to decrease the latent period between administration of carcinogen and the appearance of focal atypical cell growth (Barthold and Beck, 1980). More recently, infection has been incriminated in immune dysfunction, poor reproductive performance, and failure to thrive in T cell receptor transgenic mice (Maggio-Price *et al.*, 1998). It may also inhibit cytokine production by lymphoid cells.

e. Pseudomoniasis (Lindsey et al., 1991d)

Etiology. *Pseudomonas aeruginosa* is a motile, gram-negative rod.

Clinical signs. *Pseudomonas aeruginosa* infections are almost always silent, but immunologically compromised animals are prone to septicemia (Brownstein, 1978). *Pseudomonas aeruginosa* can, for example, cause severe or lethal infections in athymic mice. Sick mice may have equilibrium disturbances, conjunctivitis, serosanguinous nasal discharge, edema of the head, weight loss, and skin infections. Immunosuppressed mice may also develop gastrointestinal ulcers. Generalized infection is associated with severe leukopenia. Neurologic signs are rare, but there are reports of central nervous system infection.

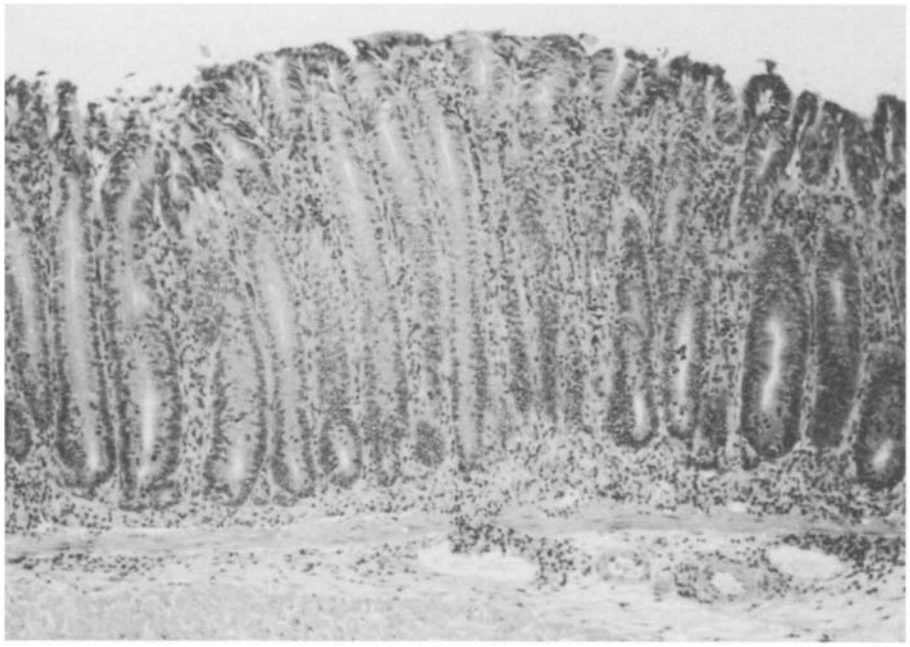

Fig. 39. Colonic hyperplasia caused by *Citrobacter rodentium.*

Chronic proliferative inflammation in the cochlea and vestibular apparatus with dissolution of surrounding bone may cause torticollis.

Epizootiology. *Pseudomonas aeruginosa* is not part of the normal flora. However, it is an opportunist that inhabits moist, warm environments such as water and skin. Once established in a host, it may be found chronically in the nasopharynx, oropharynx, and gastrointestinal tract, all sites from which additional environmental contamination or direct transmission to susceptible mice can occur.

Pathology. Pathogenic infection is most common in immunodeficient mice. Organisms enter at the squamocolumnar junction of the upper respiratory tract and, in some cases, the periodontal gingiva. Bacteremia is followed by necrosis or abscess formation in liver, spleen, or other tissues. If otitis media occurs, the tympanic bullae may contain green suppurative exudate. The bowel may be distended with fluid, and gastrointestinal ulceration has been reported.

Diagnosis. Infection is diagnosed on the basis of history (e.g., immune dysfunction), clinical signs, lesions, and isolation of *P. aeruginosa* from affected mice. Carrier mice can be detected either by nasal culture or by placing bottles of sterile, nonacidified, nonchlorinated water on cages for 24–48 hr and then culturing the sipper tubes.

Differential diagnosis. Pseudomoniasis must be differentiated from other bacterial septicemias that may occur in immunodeficient mice. These include, but are not limited to, corynebacteriosis, salmonellosis, colibacillosis, staphylococcosis, and Tyzzer's disease.

Prevention and control. Infection can be prevented by acidification or hyperchlorination of the drinking water (Homberger *et al.,* 1993). These procedures will not, however, eliminate established infections. Entry of infected animals can be prevented by surveillance of commercially procured colonies. Maintenance of *Pseudomonas*-free animals usually requires barrier-quality housing and husbandry.

Research complications. *Pseudomonas* infection is not a substantial threat to immunocompetent mice but can complicate experimental studies by causing fatal septicemia in immunodeficient mice. Virus infections that alter host defense mechanisms, such as cytomegalovirus, may enhance susceptibility to pseudomoniasis.

f. Pasteurella pneumotropica infection (Lindsey et al., 1991e)

Etiology. *Pasteurella pneumotropica* is a short, gram-negative rod.

Clinical signs. Many early observations concerning the pathogenicity of *P. pneumotropica* are questionable because they were made on colonies of mice with varying levels of bacterial and viral contamination. Infection is usually asymptomatic. Therefore, *P. pneumotropica* is most properly viewed as an opportunistic pathogen. Studies of experimental *P. pneumotropica* suggest that it may complicate pneumonias due to *Mycoplasma pulmonis* or Sendai virus. It also has been associated with suppurative or exudative lesions of the eye, conjunctiva, skin, mammary glands, and other tissues, especially in immunodeficient mice or in mice with a predisposing primary infection.

Epizootiology. *Pasteurella pneumotropica* is a ubiquitous inhabitant of the skin, upper respiratory tract, and gastrointestinal tract of mice. Litters from infected dams can become infected during the first week after birth.

Pathology. Infections can cause suppurative inflammation, which may include abscessation. Dermatitis, conjunctivitis, dacryoadenitis, panophthalmitis, mastitis, and infections of the bulbourethral glands have been attributed to *P. pneumotropica.* Preputial and orbital abscesses also occur, especially in athymic mice. Its role in metritis is unclear, but it has been cultured from the uterus, and there is some evidence that it may cause abortion or infertility. Cutaneous lesions can occur without systemic disease. They include suppurative lesions of the skin and subcutaneous tissues of the shoulders and trunk.

Diagnosis. Diagnosis requires isolation of the organism on standard bacteriological media. However, infection can be detected serologically by ELISA (Wullenweber-Schmidt, 1988; Boot *et al.,* 1995a,b). PCR assays also are available (Wang *et al.,* 1996; Weigler *et al.,* 1996).

Differential diagnosis. Suppurative lesions in mice may be caused by other bacteria, including *Staphylococcus, Streptococcus, Corynebacterium, Klebsiella,* and *Mycoplasma.*

Treatment. Antibiotic therapy has not been highly successful, although a recent report indicates that enrofloxacin (25.5–85 mg/kg) in the drinking water for 2 weeks may be effective in eliminating infection (Goelz *et al.,* 1996).

Prevention and control. Because *P. pneumotropica* is an opportunistic organism, it should be excluded from colonies containing immunodeficient mice and from breeding colonies. Achieving this goal will normally require barrier housing supported by sound microbiological monitoring. Rederivation should be considered to eliminate infection in circumstances where infection presents a potential threat to animal health or experimentation. Additionally, prophylactic administration of

trimethoprim sulfa (50–60 mg/kg) in the drinking water has been shown to prevent infection in immunodeficient mice (J. D. Macy, personal communication, 2000).

Research complications. Clinically severe infection in immunodeficient mice is the major complication.

g. *Helicobacteriosis*

Helicobacteriosis appears to be a common infection of laboratory mice. It is caused by a growing list of organisms that vary in clinical, pathologic, and epidemiologic significance (Fox and Lee, 1997). Because recognition and investigation of helicobacteriosis is relatively new, many important questions about the impact on mice remain to be answered. *Helicobacter hepaticus* infection is emphasized here, because it is among the most prevalent causes of helicobacteriosis and has been studied more extensively than other murine enterohepatic *Helicobacter* spp. (Fox *et al.,* 1994; Ward *et al.,* 1994). However, current information about other murine helicobacters is summarized in the concluding section.

Etiology. Helicobacter is a gram-negative, microaerophilic, curved to spiral-shaped organism that has been isolated from the gastrointestinal mucosa of many mammals, including humans and mice. To date, the genus includes 20 formally named *Helicobacter* spp. assigned on the basis of 16S rRNA analysis complemented by biochemical and morphological characteristics (Fox, 2000). The organisms can be grown on freshly prepared antibiotic impregnated blood agar or in broth supplemented with fetal bovine serum in a microaerobic atmosphere (5% CO_2, 90% N_2, 5% H_2) (Fox and Lee, 1997).

Eight *Helicobacter* species have been isolated from laboratory rodents. Species isolated from mice include *H. hepaticus, H. bilis* (which also infects rats), *H. muridarum, "H. rappini,"* and *H. rodentium* (named formally), and *H. 'typhlonius'* has been recently named (Fox and Lee, 1997; Franklin, *et al.,* 2001). These organisms are most commonly urease-, catalase-, and oxidase-positive. However, *H. rodentium, H. 'typhlonicus,'* and another novel *Helicobacter* sp. are urease-negative.

Clinical signs. Helicobacteriosis in adult immunocompetent mice is usually asymptomatic. Liver enzymes are elevated in *H. hepaticus*–infected A/J mice (Fox *et al.,* 1996). Infection of immune-dysregulated mice with *H. hepaticus* can cause inflammatory bowel disease, which may present as rectal prolapse and/or diarrhea.

Epizootiology. Recent surveys and anecdotal evidence suggest that helicobacteriosis is widespread among conventional and barrier-maintained mouse colonies (Shames *et al.,* 1995; Fox *et al.,* 1998). Furthermore, *H. hepaticus* (and probably other helicobacters) can persist in the gastrointestinal tract, particularly the cecum and colon, and is readily detected in feces. These results indicate that transmission occurs primarily by the fecal–oral route and imply that carrier mice can spread infection chronically in enzootically infected colonies.

Pathology. Helicobacter spp. colonize the crypts of the lower bowel, where, depending on host genotype, the organisms can be pathogenic or nonpathogenic. *Helicobacter hepaticus,* for example, can cause inflammation in the gastrointestinal tract, which is expressed as inflammatory bowel disease (IBD) in immunodeficient mice or typhlitis in A/J mice (Fig. 40) (Ward *et al.,* 1996). Thickening of the cecum and large bowel develops because of proliferative typhlitis, colitis, and proctitis, which can occur without coincident hepatitis.

Helicobacters also can cause liver disease. Bacterial translocation is thought to occur and results in colonization of the liver and progressive hepatitis (Fig. 41). It is characterized by angiocentric nonsuppurative hepatitis and hepatic necrosis. Inflammation originates in portal triads and spreads to adjacent hepatic parenchyma. Hepatic necrosis also may occur adjacent to intralobular venules, which can contain microthrombi. Additionally, phlebitis may affect central veins. This lesion has been linked to the presence of organisms by silver stains and electron microscopy. Age-related hepatocytic proliferation can develop in infected livers, a response that is more pronounced in male mice than in female mice (Fox *et al.,* 1996). This lesion may increase susceptibility to hepatomas and hepatocellular carcinomas among aged male A/JCr and B6C3F1 mice from infected colonies. An increased incidence of hepatic haemangiosarcomas also has been noted in *H. hepaticus*–infected male B6C3F1 mice. In this context, A/JCr, C3H/HeNCr, and SJL/NCr mice are susceptible to hepatitis, whereas C57BL/6 mice are resistant (Ward *et al.,* 1994). The finding of severe liver disease and tumor induction in B6C3F1 mice infected with *H. hepaticus* infers that genetic susceptibility to *H. hepaticus*–induced neoplasia has a dominant pattern of inheritance. Recent studies with *H. hepaticus* in recombinant inbred mice also indicate that disease susceptibility has multigenetic properties (Hailey, 1998; Fox and Lee, 1997; Ihrig *et al.,* 1999).

Diagnosis. Rapid generic diagnosis can be accomplished by PCR detection of the highly conserved 16S rRNA region of the *Helicobacter* genome in feces or tissues, using suitable oligonucleotide primers (Shames *et al.,* 1995). However, PCR does not differentiate among *H. hepaticus, H. bilis, H. 'typhlonicus,' H. muridarum,* and *"H. rappini."* Molecular speciation can be accomplished by retriction fragment length polymorphism analysis of the PCR product. This procedure requires suitable skill and experience to avoid technological pitfalls and should be performed by qualified laboratories. An IgG ELISA using outer membrane protein as the antigen shows promise for serological diagnosis. As noted above, helicobacters can be isolated

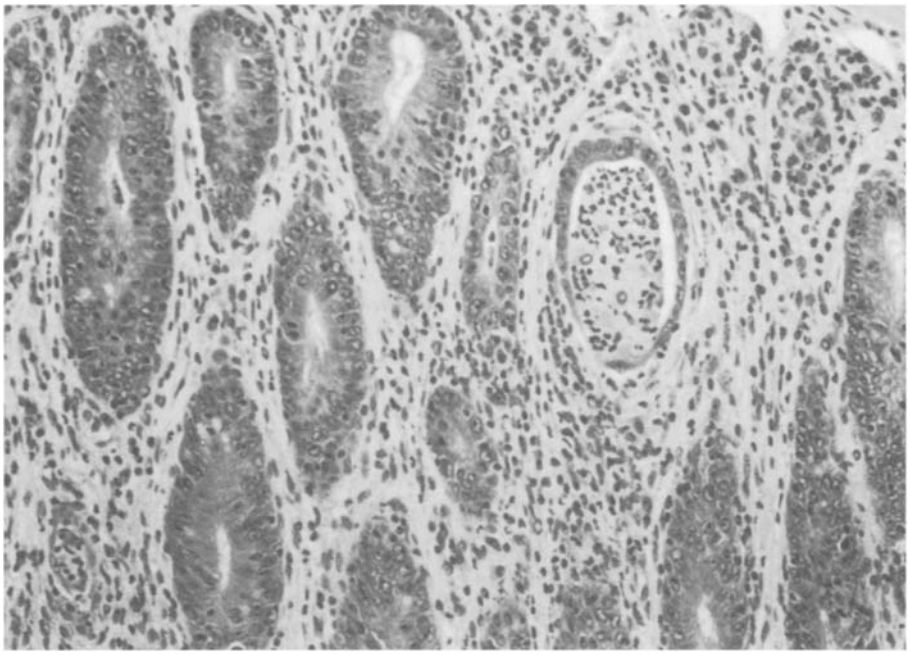

Fig. 40. Inflammatory bowel disease associated with *Helicobacter hepaticus* in a SCID mouse reconstituted with $CD45RB^{high}$ $CD4^+$ T cells.

on antibiotic-impregnated blood agar under microaerobic conditions and can then be speciated biochemically. Isolation of *H. hepaticus* from feces should be preceded by passing slurried samples through a 0.45 m filter before plating. If infection with larger helicobacters (*H. bilis, "H. rappini"*) is suspected, filtration at 0.65 μm is preferred. Helicobacters grow slowly and require prolonged incubation of cultures (up to 3 weeks) before they can be deemed negative. Signs (rectal prolapse) and lesions (hepatitis, typhlocolitis), depending on host genotype, can be suggestive of infection. Histopathological examination should include silver stains, especially of liver, to attempt to visualize spiral or curved organisms (Fox and Lee, 1997).

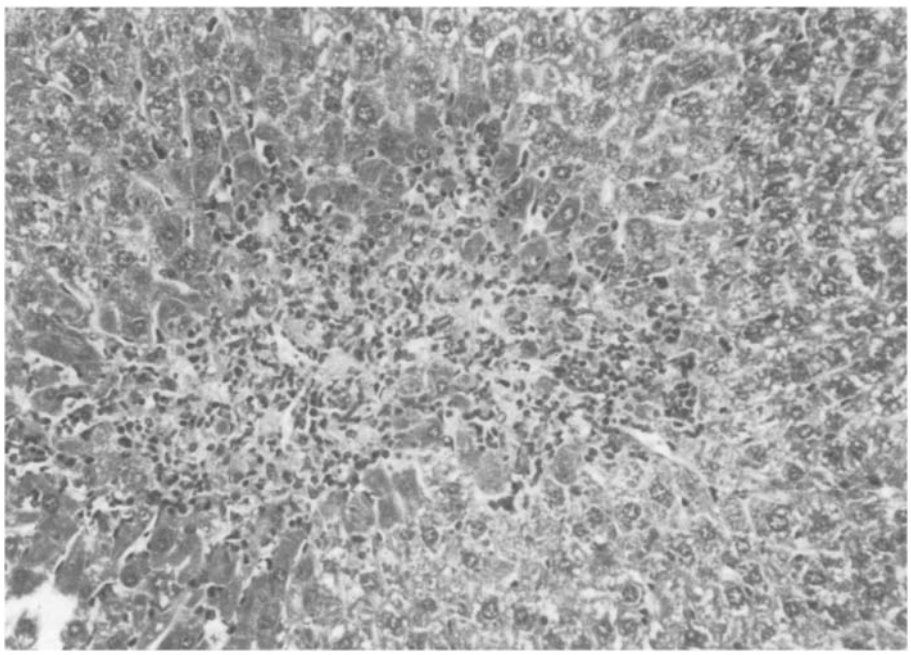

Fig. 41. Hepatitis caused by *Helicobacter hepaticus* in an A/JCr male mouse.

Differential diagnosis. Clinically apparent helicobacteriosis must be differentiated from other gastrointestinal or hepatic infections of mice. Coronavirus infection, *Clostridium piliforme,* and *Salmonella* spp. can cause enterocolitis and/or hepatitis. *Citrobacter rodentium* also causes colonic hyperplasia, which can present as rectal prolapse.

Infections caused by other helicobacters of mice. Helicobacter bilis has been isolated from the livers and intestines of aged mice and experimentally induces inflammatory bowel disease (IBD) in SCID mice as does *H. hepaticus. Helicobacter muridarum* colonizes the ileum, cecum, and colon. It appears to be nonpathogenic, although it can colonize the stomach of mice and induce gastritis under certain circumstances. *Helicobacter "rappini"* has been isolated from the feces of mice without clinical signs. *Helicobacter rodentium* also colonizes the intestine and may be a component of normal flora. A dual infection of *H. bilis* and *H. rodentium* was noted in a natural outbreak of IBD in immunocompromised mice (Shomer *et al.,* 1998). A novel urease negative helicobacter, which has been named *H. 'typhlonius',* causes IBD in $IL10^{-/-}$ and SCID mice (Fox *et al.,* 1999; Franklin *et al.,* 1999, 2001).

Prevention and control. Eradication of infection from small numbers of mice, such as quarantine groups, can be achieved by standard rederivation or intensive antibiotic therapy. The best results have been obtained by triple therapy with amoxicillin, metranidazole, and bismuth given for 2 weeks (Foltz *et al.,* 1996). This strategy requires repeated daily gavage rather than administration in drinking water, but it has successfully eliminated *H. hepaticus* from naturally infected mice. Wide-scale, eradication of enzootic helicobacteriosis can be expensive and time-consuming, without guarantee of success. Careful husbandry procedures can limit infection within a colony (Whary *et al.,* 2000). Therefore, strategies have to be weighed carefully against risks of enzootic infection for the health and use of mice. By contrast, infection should be avoided in immunodeficient mice, including genetically engineered mice with targeted or serendipitous immune dysfunction. Lastly, the outcome of opportunistic helicobacteriosis has not been thoroughly examined. This condition could occur during simultaneous infection with two or more *Helicobacter* species or during combined infection with an intestinal virus (e.g., coronavirus) and *Helicobacter* spp. If highly valuable animals are exposed, antibiotic therapy or rederivation may be warranted.

Research complications. Chronic inflammation of the liver and or gastrointestinal tract may be injurious to health. Additionally, it may impede the development and assessment of noninfectious disease models, such as IBD models in mice with targeted deletions in T-lymphocyte receptors (Fox *et al.,* 2000). *Helicobacter hepaticus* infections provoke a strong Th1 proinflammatory response, which may perturb other immunological responses. *Helicobacter hepaticus* infection also has been incriminated as a cofactor or promoter in the development of hepatic neoplasia in A/JCr and B6C3F1 mice (Hailey, *et al.,* 1998; Fox, *et al.,* 1998).

h. Salmonellosis (Ganaway, 1982; Lindsey et al., 1991f)

Etiology. There are approximately 2400 known serotypes of *Salmonella choleraesuis,* with serotypes *S. enteritidis* and *S. typhimurium* constituting the most frequent isolates from mice. *Salmonella enteritidis* is a motile, gram-negative rod that rarely ferments lactose.

Clinical signs. Acute infection is especially severe in young mice (Casebolt and Schoeb, 1988). It is characterized by anorexia, weight loss, lethargy, dull coat, humped posture, and occasionally conjunctivitis. Gastroenteritis is a common sign, but feces may remain formed. Subacute infection can produce distended abdomens from hepatomegaly and splenomegaly. Chronic disease is expressed as anorexia and weight loss. Enzootic salmonellosis in a breeding colony can produce epsiodic disease with alternating periods of quiescence and high mortality. The latter can be associated with diarrhea, anorexia, weight loss, roughened hair coat, and reduced production.

Epizootiology. Modern production and husbandry methods have reduced the importance of salmonellosis as a natural infection of mice. However, the organisms are widespread in nature. Therefore, cross-infection from other species or from feral mice remains a potential hazard. Salmonellas are primarily intestinal microorganisms that can contaminate food and water supplies. Infection occurs primarily by ingestion. Vermin, birds, feral rodents, and human carriers are potential sources of infection. Other common laboratory species such as nonhuman primates, dogs, and cats also can serve as carriers. Conversely, murine salmonellosis presents a zoonotic hazard to people.

The induction and course of infection are influenced by the virulence and dose of the organism; route of infection; host sex and genetic factors; nutrition; and intercurrent disease. Suckling and weanling mice are more susceptible to disease than mature mice. Immune deficiency, exposure to heavy metals, and environmental factors such as abnormal ambient temperatures can increase the severity of disease. Nutritional iron deficiency has an attenuating effect on *Salmonella* infection in mice, whereas iron overload appears to promote bacterial growth and enhance virulence. Resistance to natural infection is increased by the presence of normal gastrointestinal microflora. Resistance to infection also can be an inherited trait among inbred strains. Among the most important considerations is that mice that recover from acute infection can become asymptomatic carriers and a chronic source of contamination from fecal shedding.

Pathology. The virulence of *S. enteritidis* depends on its ability to penetrate intestinal walls, enter lymphatic tissue, multiply, and disseminate. Organisms reach Peyer's patches within 12 hr after inoculation and spread quickly to the mesenteric lymph nodes. Bacteremia results in spread to other lymph nodes, spleen, and liver within several days. In chronic infections, organisms persist in the spleen and lymph nodes as well as in the liver and gallbladder and from the latter are discharged into the intestinal contents. Bacteria reaching the intestine can reinvade the mucosa and can be shed intermittently in the feces for months. *Salmonella enteritidis* infection also has been associated with chronic arthritis.

Acute deaths may occur without gross lesions, but visceral hyperemia, pale livers, and catarrhal enteritis are more common. If mice survive for up to several weeks, the intestine may be distended and reddened, while the liver and spleen are enlarged and contain yellow-gray foci of necrosis. Affected lymph nodes are also enlarged, red, and focally necrotic. Focal inflammation can develop in many organs, including the myocardium (Percy and Barthold, 2001b).

Histologic lesions reflect the course of disease and the number of bacteria in affected tissues. During acute infection, necrotic foci are found in the intestine, mesenteric lymph nodes, liver, and spleen. Neutrophilic leukocytes and histiocytes accumulate in lymphoid tissues. Thrombosis from septic venous embolism may occur, especially in the liver. Granulomatous lesions are particularly characteristic of chronic salmonellosis, especially in the liver.

Diagnosis. Diagnosis is based on isolation of salmonellas together with documentation of compatible clinical signs and lesions. In mice with systemic disease, bacteria may persist in the liver and spleen for weeks. During acute stages, bacteria can also be isolated from the blood. Asymptomatically infected animals can be detected by fecal culture using selective enrichment media, but culture of the mesenteric lymph nodes may be more reliable because fecal shedding can be intermittent. Isolates can be speciated with commercial serotyping reagents. Alternatively, isolates can be sent to a reference laboratory for confirmation. Antibodies to salmonellas can be detected in the serum of infected mice by an agglutination test. However, this method is not entirely reliable, because serological cross-reactivity is common even among bacteria of different genera.

Differential diagnosis. Salmonellosis must be differentiated from other bacterial diseases, including Tyzzer's disease, pseudomoniasis, corynebacteriosis, murine colonic hyperplasia, and pasteurellosis. Viral infections that cause enteritis or hepatitis must also be considered, especially infections caused by coronavirus, ectromelia virus, and reoviruses. Among noninfectious conditions, mesenteric lymphadenopathy is an aging-associated lesion in mice and is not indicative of chronic salmonellosis.

Prevention and control. Salmonellosis can be prevented by proper husbandry and sanitation. Contact between mice and potential carriers, such as nonhuman primates, dogs, and cats, should be prevented. Diets should be cultured periodically to check for inadvertent contamination. Contaminated colonies should be replaced to eliminate infection and its zoonotic potential.

Research complications. Apart from the clinical manifestations, the zoonotic potential for salmonellosis is a major concern. This includes transmission among laboratory species, but especially between mice and the people working with them.

i. Streptobacillosis (Lindsey et al., 1991g)

Etiology. Streptobacillus moniliformis is a nonmotile, gram-negative, pleomorphic rod that can exist as a nonpathogenic L phase variant *in vivo.* However, it can revert to the virulent bacillus form.

Clinical signs. Streptobacillosis generally has an acute phase with high mortality, followed by a subacute phase and finally a chronic phase that may persist for months. Signs of acute disease include a dull, damp hair coat and keratoconjunctivitis. Variable signs include anemia, diarrhea, hemoglobinuria, cyanosis, and emaciation. Cutaneous ulceration, arthritis, and gangrenous amputation may occur during chronic infection. The arthritis can leave joints deformed and ankylosed. Hindlimb paralysis with urinary bladder distention, incontinence, kyphosis, and priapism may occur if vertebral lesions impinge on motor nerves. Breeding mice may have stillbirths or abortions.

Epizootiology. Streptobacillosis has historical importance as a disease of mice, but modern husbandry, production, and health care strategies have reduced its impact dramatically (Wullenweber, 1995). Asymptomatic, persistently infected rats are the most likely source of dissemination to mice, but mouse-to-mouse transmission can follow. Transmission may occur from aerogenic exposure, bite wounds, or contaminated equipment, feed, or bedding. *Streptobacillus moniliformis* also is pathogenic for humans, causing rat bite fever (Haverhill fever).

Pathology. During acute disease, necrotic lesions develop in thoracic and abdominal viscera, especially in liver, spleen, and lymph nodes. Histological lesions include necrosis, septic thrombosis of small vessels, acute inflammation, fibrin deposition, and abscesses. Chronically infected mice may develop purulent polyarthritis because of the organism's affinity for joints.

Diagnosis. Diagnosis depends on clinical and pathological evidence of septicemia and isolation of the organism. The organism has been recovered from joint fluid as long as 26 months

after infection. Isolation from chronic lesions requires serum-enriched medium.

Differential diagnosis. Clinical signs must be differentiated from septicemic conditions, including mousepox, Tyzzer's disease, corynebacteriosis, salmonellosis, mycoplasmosis, pseudomoniasis, and traumatic lesions.

Prevention and control. Control is based on exclusion of wild rodents or carrier animals such as latently infected laboratory rats. Bacterins and antibiotic therapy are not adequately effective. The potential for cross-infection is a reason not to house rats and mice in the same room.

Research complications. Infection can be disabling or lethal in mice and has zoonotic potential for humans.

j. Corynebacteriosis (Lindsey et al., 1982; Weisbroth, 1994)

Etiology. Corynebacteria are short gram-positive rods. *Corynebacterium kutscheri* is the cause of pseudotuberculosis in mice and rats. *Corynebacterium bovis* has been associated with hyperkeratosis, especially in immunodeficient mice (Clifford *et al.,* 1995; Scanziani *et al.,* 1998).

Clinical signs. Corynebacterium kutscheri infection is often asymptomatic in otherwise healthy mice. Active disease is precipitated by immunosuppression or environmental stresses and is expressed as an acute illness with high mortality or a chronic syndrome with low mortality. Clinical signs include inappetance, emaciation, rough hair coat, hunched posture, hyperpnea, nasal and ocular discharge, cutaneous ulceration, and arthritis. *Corynebacterium bovis* infection causes hyperkeratotic dermatitis. It is characterized by scaly skin, which is accompanied by alopecia in haired mice. Severe infection may cause generalized weakness (Clifford *et al.,* 1995). Corynebacterial keratoconjunctivitis has been reported in aged C57BL/6 mice (McWilliams *et al.,* 1993).

Epizootiology. The epizootiology of corynebacteriosis is unclear. Asymptomatically infected animals are presumed to harbor organisms in the upper alimentary tract, colon, and/or respiratory tract and regional lymph nodes. Other sites include the middle ears and preputial glands. Therefore, transmission by multiple routes is possible, including the fecal–oral route. Resistance to infection appears to be under genetic control in some mouse strains. Rats are susceptible to *C. kutscheri,* so cross-infection to mice may occur. *Corynebacterium bovis* infection can be transmitted from mouse to mouse by contact.

Pathology. Lesions caused by *C. kutscheri* develop from hematogenous spread to various internal organs and appear as gray-white nodules in kidney, liver, lung, and other sites (Percy and Barthold, 2001c). Cervical lymphadenopathy and arthritis of the carpometacarpal and tarsometatarsal joints also may occur. Septic, necrotic lesions often contain caseous material or liquefied pus. Histologic lesions are characterized by coagulative or caseous necrosis bordered by intense neutrophilic infiltration. Colonies of gram-positive organisms can usually be demonstrated in caseous lesions. Mucopurulent arthritis of carpal, metacarpal, tarsal, and metatarsal joints are related to bacterial colonization of synovium accompanied by necrosis, cartilage erosion, ulceration, and eventually ankylosing panarthritis. *Corynebacterium kutscheri* is not a primary skin pathogen, but skin ulcers or fistulas follow bacterial embolization and infarction of dermal vessels. Subcutaneous abscesses have also been reported.

Hyperkeratotic dermatitis caused by *C. bovis* is characterized grossly by skin scaliness and alopecia. Microscopically, skin lesions consist of prominent acanthosis and moderate hyperkeratosis accompanied by mild nonsuppurative inflammation (Fig. 42). Hyperkeratosis is typically more severe in glabrous mice than in haired mice. Organisms can be demonstrated in hyperkeratotic layers by Gram stain.

Diagnosis. Diagnosis depends on isolation and identification of the causative bacteria. Additionally, organisms compatible with *C. kutscheri* are usually demonstrable with tissue Gram stains on lesions from clinically apparent cases. Agglutination serology is available, and immunofluorescence, immunodiffusion, and ELISA tests have been reported (Boot *et al.,* 1995b).

Differential diagnosis. The caseous nature of *C. kutscheri*–induced lesions helps separate them from necrotic changes or abscesses caused by other infectious agents of mice. Thus, they can be differentiated from streptococcosis, mycoplasmosis, and other septicemic bacterial infections in which caseous necrosis does not occur. Because mice can sustain natural infections with *Mycobacterium avium,* histochemical techniques for acid-fast bacilli and appropriate culture methods for mycobacteria should be considered if nodular inflammatory lesions of the lung are detected. Hyperkeratotic dermatitis caused by *C. bovis* must be differentiated from scaly skin caused by low humidity in glabrous mice.

Prevention and control. Because clinically apparent corynebacteriosis occurs sporadically, treatment and control are difficult. Antibiotic therapy is probably inhibitory or suppressive, but not preventive or curative. Culling of clinically ill animals may be useful for conventional colonies but is of little use for immunodeficient mice or for mice that will endure significant stress during experimentation. Replacing or rederiving infected colonies in a specific pathogen-free environment can be effective in eliminating and preventing reinfection.

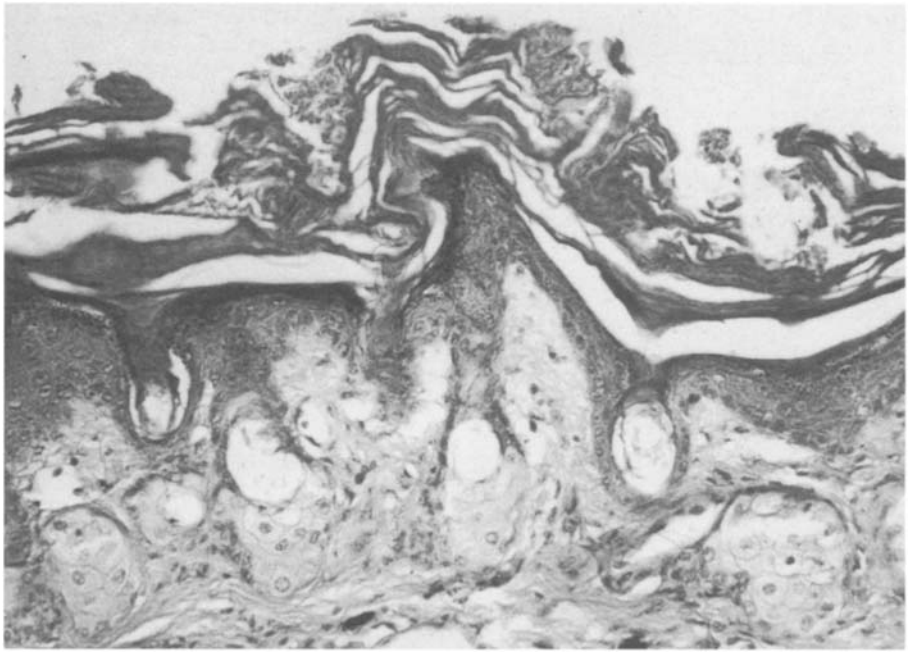

Fig. 42. Hyperkeratosis associated with *Corynebacterium bovis* infection.

Research complications. Corynebacteriosis is a potential threat for morbidity and mortality, especially among immunodeficient mice. Dermatologic disease in suckling mice can be fatal but is less severe and transient in weanling mice.

k. Staphylococcosis (Besch-Williford and Wagner, 1982; Lindsey et. al., 1991h; Shimizu, 1994)

Etiology. Staphylococci are gram-positive organisms that commonly infect skin and mucous membranes of mice and other animals. The two most frequently encountered species are *Staphylococcus aureus,* which can be highly pathogenic, and *S. epidermidis,* which is generally nonpathogenic. Species subtypes are identified by phage typing and biochemistry. Pathogenic staphylococci are typically coagulase-positive.

Clinical signs. Staphylococcosis causes suppurative dermatitis in mice. Some evidence suggests that staphylococci can produce primary cutaneous infections, but they are more likely opportunistic organisms that induce lesions after contamination of skin wounds. Eczematous dermatitis develops primarily on the face, ears, neck, shoulders, and forelegs and can progress to ulcerative dermatitis, abscessation (including botryomycotic granulomas), and cellulitis. Because lesions are often pruritic, scratching causes additional trauma and autoinoculation. Staphylococcal infection in the genital mucosa of males may produce preputial gland abscesses. These occur as firm, raised nodules in the inguinal region or at the base of the penis and may rupture to spread infection to surrounding tissues. Male mice also may develop septic balanoposthitis secondary to penile self-mutilation. Retrobulbar abscesses caused by *S. aureus* are frequently noted in athymic mice.

Epizootiology. Staphylococci are ubiquitous and can be carried on the skin and in the nasopharnx and gastrointestinal tract. They also can be cultured from cages, room surfaces, and personnel. The prevalence of staphylococcal dermatitis appears to be influenced by host genotype, the overall health of the animal, and the degree of environmental contamination with *Staphylococcus* spp. C57BL/6, C3H, DBA, and BALB/c mice seem to be the most susceptible strains. Age may also influence susceptibility, with young mice being more susceptible than adults. Immunodeficient mice (e.g., athymic mice) contaminated with staphylococci often develop abscesses or furunculosis (Fig. 43). As noted above, behavioral dysfunction resulting in self-mutilation, including scratching and trichotillomania, is a likely predisposing factor. Once virulent staphylococci contaminate the environment, colonization of the gastrointestinal tract can occur and produce a carrier state. Phage typing can help to determine the source of infection. Human phage types of staphylococci can infect mice, but the zoonotic importance of this connection is not clear.

Pathology. Gross lesions are typified by suppurative or ulcerative dermatitis involving the head and neck but may extend to the shoulders and forelegs (Percy and Barthold, 2001d). Superficial or deep abscesses may occur in conjunction with dermatitis or separately, as, for example, in the external male

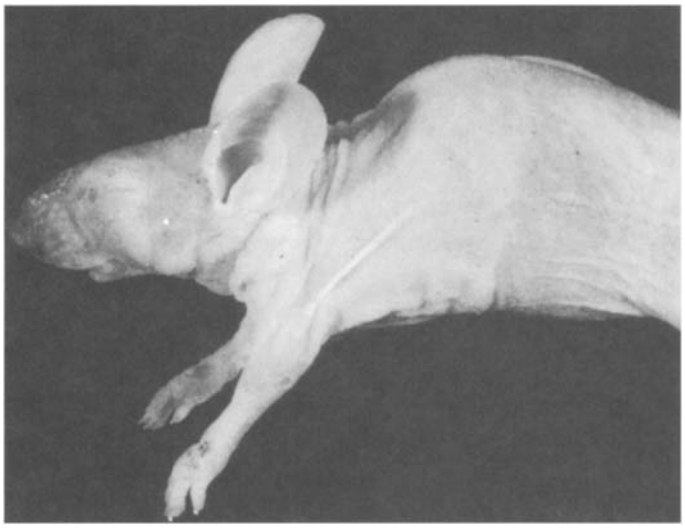

Fig. 43. Furunculosis in an athymic mouse.

genitalia. Histologically, acute skin infections result in ulceration with neutrophils in the dermis and subcutis. There also may be regional lymphadenitis. Chronic lesions contain lymphocytes, macrophages, and fibroblasts. Deep infections appear as coalescing botryomycotic pyogranulomas with necrotic centers containing bacterial colonies.

Diagnosis. Diagnosis is made by documenting gross and histological lesions, including Gram staining of suspect tissues, complemented by isolation of gram-positive, coagulase-positive cocci.

Differential diagnosis. Staphylococcosis must be differentiated from other suppurative infections of mice, including pasteurellosis, streptococcosis, corynebacteriosis, and pseudomoniasis. Ectoparasitism, fight wounds, and self-mutilation per se should also be considered.

Prevention, control, and treatment. Removal of affected animals, sterilization of food and bedding, and frequent changing of bedding may limit or reduce transmission. Affected animals may be helped by nail trimming to reduce self-inflicted trauma. Conditions that facilitate aggressive or self-mutilating behavior should be avoided.

Research complications. Staphylococcosis can cause illness and disfigurement in mice. Immunodeficient mice are at increased risk for these conditions.

l. Streptococcosis (Lindsey et al., 1991j; Nakagawa and Weyant, 1994)

Etiology. Streptococci are ubiquitous gram-positive organisms. Most streptococcal infections in laboratory mice are caused by β-hemolytic organisms in Lancefield's group C, but epizootics caused by group A and group D streptococci have occurred, and group G organisms have been isolated occasionally. However, α-hemolytic streptococci can cause systemic disease in SCID mice, and group B *Streptococcus* infection has been reported to cause meningoencephalitis in athymic mice (Schenkman *et al.,* 1994). Additionally, *Streptococcus equisimilis* has been isolated from visceral abscesses of immunocompetent mice (Greenstein *et al.,* 1994).

Clinical signs and pathology. Cutaneous infections can cause ulcerative dermatitis over the trunk, which may appear gangrenous, whereas systemic infections may be expressed as conjunctivitis, rough hair coat, hyperpnea, somnolescence, and emaciation. Systemic lesions reflect hematogenous dissemination and include abscessation, endocarditis, splenomegaly, and lymphadenopathy (Percy and Barthold, 2001e). Streptococcal cervical lymphadenitis can lead to fistulous drainage to the neck complicated by ulcerative dermatitis. Infection with α-hemolytic streptococci can cause inflammatory lesions affecting kidney and heart.

Epizootiology. Mice can carry streptococci asymptomatically in their upper respiratory tracts. Lethal epizootics can occur, but factors leading to clinical disease are unknown, although some infections may be secondary to wound contamination.

Diagnosis. Diagnosis and differential diagnosis depend on isolation of organisms from infected tissues, combined with histopathologic confirmation.

Research complications. Immunodeficient mice are at increased risk for streptococcosis.

m. Colibacillosis

Escherichia coli is a small gram-negative rod that is a normal inhabitant of the mouse intestine. Infection is considered nonpathogenic in immunocompetent mice. However, hyperplastic typhlocolitis resembling transmissible murine colonic hyperplasia has been reported in SCID mice infected with a non-lactose-fermenting *Escherichia coli* (Waggie *et al.,* 1988). Affected mice develop fecal staining and move slowly.

Gross lesions consist of segmental thickening of the colon or cecum, which may contain blood-tinged feces. Microscopically, affected mucosa is hyperplastic and may be inflamed and eroded. Diagnosis depends on demonstrating lesions and isolating non-lactose-fermenting *E. coli.* This condition must be differentiated from proliferative and inflammatory intestinal disease caused by *Citrobacter rodentium* or by enterotropic mouse hepatitis virus, especially in immunodeficient mice. Colibacillosis provides an example of the morbidity associated with a

nominally innocuous organism when it affects an immunocompromised host.

n. Klebsiellosis

Klebsiella pneumoniae is a ubiquitous gram-negative organism that is a natural inhabitant of the mouse alimentary tract. It can be pathogenic for the respiratory and urinary tract of mice after experimental inoculation but is not a significant cause of naturally occurring disease. *Klebsiella oxytoca,* among other organisms, has been found in mice with suppurative endometritis, salpingitis, and oophoritis but did not elicit this condition after experimental inoculation of mice.

o. Clostridium Infection

Clostridia are large, rod-shaped, gram-positive anaerobic bacteria. Naturally occurring clostridial infection in mice is rare. Epizootics of *Clostridium perfringens* type D infection with high mortality have been reported in a barrier colony where heavy mortality occurred in 2- to 3-week-old suckling mice. Clinical signs included scruffy hair coats, paralysis of the hindquarters, and diarrhea or fecal impaction. However, attempts to reproduce the disease experimentally with clostridia isolated from naturally infected animals were unsuccessful. *Clostridium perfringens* also has been isolated from sporadic cases of necrotizing enteritis in recently weaned mice.

p. Mycobacteriosis

Two mycobacteria are known to be pathogenic for laboratory mice: *Mycobacterium avium-intracellulare* and *M. lepraemurium.* Both are gram-positive, acid-fast, obligate intracellular bacteria.

Infection with *M. avium-intracellulare* should be considered extremely rare, with the only published report describing an episode in a breeding colony of C57BL/6 mice (Waggie *et al.,* 1983). The source of the outbreak was presumed to be drinking water. Clinical signs did not occur, but mice developed granulomatous pneumonia, which, in some mice, included Langhans' giant cells. Other lesions included microgranulomas in the liver and lymph nodes. Acid-fast bacilli were demonstrated in some lesions. Mycobacteria are widespread in water and soil. Their presence in laboratory mice would indicate a significant break in husbandry practices.

Mycobacterium lepraemurium has been isolated from healthy laboratory mice and can persist as a latent infection, but its significance is primarily historical, as a model for human leprosy. It is highly unlikely to encounter this infection in a modern, well-managed mouse colony. If clinically apparent infection does occur, it is expressed as a chronic granulomatous disease. Clinical signs include alopecia, thickening of skin, subcutaneous swellings, and ulceration of the skin. Disease can lead to death or clinical recovery. Gross lesions are characterized by nodules in subcutaneous tissues and in reticuloendothelial tissues and organs (lung, spleen, bone marrow, thymus, and lymph nodes). Lesions can also occur in lung, skeletal muscle, myocardium, kidneys, nerves, and adrenal glands. The histologic hallmark is perivascular granulomatosis with accumulation of large, foamy epithehoid macrophages (lepra cells) packed with acid-fast bacilli.

q. Proteus Infection

Proteus mirabilis is a ubiquitous gram-negative organism that can remain latent in the respiratory and intestinal tracts. Clinical disease can occur following stress or induced immunosuppression. Immunodeficient mice have a heightened susceptibility to pathogenic infection. *Proteus* has been associated with ulcerative lesions in the gastrointestinal tract of immunodeficient mice. Infected animals lose weight, develop diarrhea, and die within several weeks. If septicemia develops, suppurative or necrotic lesions, including septic thrombi, may be found in many organs, but the kidney is commonly affected. *Proteus* pyelonephritis is characterized by abscessation and scarring. Ascending lesions may occur following urinary stasis, but hematogenous spread cannot be ruled out. *Proteus mirabilis* and *Pseudomonas aeruginosa* have been isolated concomitantly from cases of suppurative nephritis or pyelonephritis. Infection in immunodeficient mice is typified by splenomegaly and focal necrotizing hepatitis. Pulmonary lesions include edema and macrophage activation. Septic thrombi can occur, however, in many tissues.

r. Leptospirosis

Leptospirosis is exceedingly rare in laboratory mice. Infection with *Leptospira interrogans* serovar *ballum* has been reported on several occasions. It is a gram-negative organism that, after a septicemic phase, establishes persistent infection in the renal tubules and is excreted in the urine. Infection is asymptomatic and causes no significant lesions. Therefore, diagnosis requires isolation of organisms in kidney culture. Serological testing should be used with caution because neonatal exposure can lead to persistent infection without seroconversion. Histologic examination of kidney using silver stains can also be attempted. Persistent murine infections associated with active shedding present a zoonotic hazard for humans; therefore infected mice should be discarded. Elimination of infection from highly valuable mice requires rederivation.

3. Rickettsial and Chlamydial Diseases (Hildebrandt, 1982)

a. Rickettsia Infection

Etiology. Two rickettsia, *Eperythrozoon coccoides* and *Hemobartonella muris,* are known to infect mice. *Eperythrozoon*

coccoides is primarily an organism of mice, whereas *H. muris* can infect mice but is more commonly associated with rats. *Eperythrozoon* occurs as a ring-shaped, coccoid, or occasionally rod-shaped organism that occurs in blood either attached to erythrocytes or free in plasma. It is enclosed by a single limiting membrane but has no cell wall and no nucleus or other membrane-bound organelles.

Clinical signs. Mice infected with *E. coccoides* may remain clinically normal or develop febrile, hemolytic anemia and splenomegaly, which can be fatal. Hepatocellular degeneration and multifocal necrosis have been recorded in acute infections. Rickettsial infections are long-lived and are expressed clinically in one of two ways: acute febrile anemia and latent or asymptomatic infection that can be reactivated by splenectomy. The carrier state may be lifelong.

Epizootiology. The primary natural vector of *E. coccoides,* historically, was the mouse louse, *Polyplax serrata.* Therefore infection was associated with primitive housing and husbandry conditions that no longer occur in modern vivaria. Although the risks for infection have been reduced substantially by modern animal care procedures, *E. coccoides* can be transmitted to mice from contaminated biological products such as transplantable tumors or blood plasma.

Diagnosis. Splenectomy or inoculation of test material into splenectomized mice is the most sensitive means to detect *E. coccoides* infection. These procedures provoke rickettsemia, usually within 2–4 days. Because rickettsemia may be transient, blood smears stained by the Romanowsky or indirect immunofluorescence procedure should be prepared every 6 hr beginning at 48 hr after splenectomy of index animals or inoculation of test specimens into splenectomized animals to assure that rickettsemia is not missed.

Prevention and control. Treatment of *E. coccoides* infection is not practical. Control is based on elimination of lice and or rederivation of infected stock. If replacement animals are readily available, euthanasia is a more prudent course. Suspect biological materials destined for animal inoculation should be checked for rickettsial contamination by inoculation of splenectomized mice.

Research complications. Asymptomatic infection can be reactivated by irradiation, immunosuppressive therapy, or intercurrent disease. Conversely, *E. coccoides* may potentiate coincident viral infections in mice. This effect has been clearly demonstrated for mouse coronavirus and has been suspected for lymphocytic choriomeningitis virus and LDV. Active infection also may suppress interferon production.

b. Chlamydia Infection

Chlamydia trachomatis is an intracellular organism that produces glycogen-positive intracytoplasmic inclusions (elementary bodies). *Chlamydia trachomatis* causes ocular and urogenital disease in humans. However, at least one strain, the so-called Nigg agent, is thought to be responsible for a historically noteworthy infection in mice. Natural infections are typically asymptomatic but persistent. Severe acute infection is characterized by ruffled fur, hunched posture, and labored respiration due to interstitial pneumonitis and leads to death in 24 hr. Mice dying more slowly may develop progressive emaciation and cyanosis of the ears and tail.

4. Mycotic Diseases

a. Pneumocystosis

Etiology. Pneumocystis carinii (*Pc*) is a common opportunistic organism of laboratory mice and other mammals. It had been classified initially as a protozoon, but contemporary molecular analysis of its nucleic acids and proteins places it among the fungi.

Clinical signs. Infection is asymptomatic in immunocompetent mice. However, it can be clinically severe in immunodeficient mice, because an adequate complement of functional T lymphocytes is required to suppress infection (Roths *et al.,* 1990; Shultz and Sidman, 1987; Walzer *et al.,* 1989; Weir *et al.,* 1986). Infection proceeds slowly, but relentlessly in immunodeficient mice leading to clinical signs of pneumonia, usually within several months. Primary signs include dyspnea and hunched posture, which may be accompanied by wasting and scaly skin. Severe cases, such as those that occur in advanced disease in SCID mice, may be fatal.

Epizootiology. Pc is a ubiquitous organism that is often present as a latent infection. Although firm prevalence data are not available, because detection methods are not simple to apply, one should assume that infection is present in mouse colonies unless ruled out by extensive surveillance. *Pc* isolates from different species (e.g., mice and rats) differ antigenically, but interspecies transmission can occur. *Pc* infection also occurs in human beings, but transmission between rodents and human beings has not been documented. *Pc* is transmitted aerogenically and establishes persistent, quiescent infection in the lungs of immunocompetent mice. Prenatal infection has not been demonstrated.

Pathology. Pc is normally not pathogenic but can be activated by intercurrent immunosuppression. Activation fills the lung with trophic and cystic forms. Gross lesions occur in the lungs, which are often rubbery and fail to deflate (Fig. 44). Histopatho-

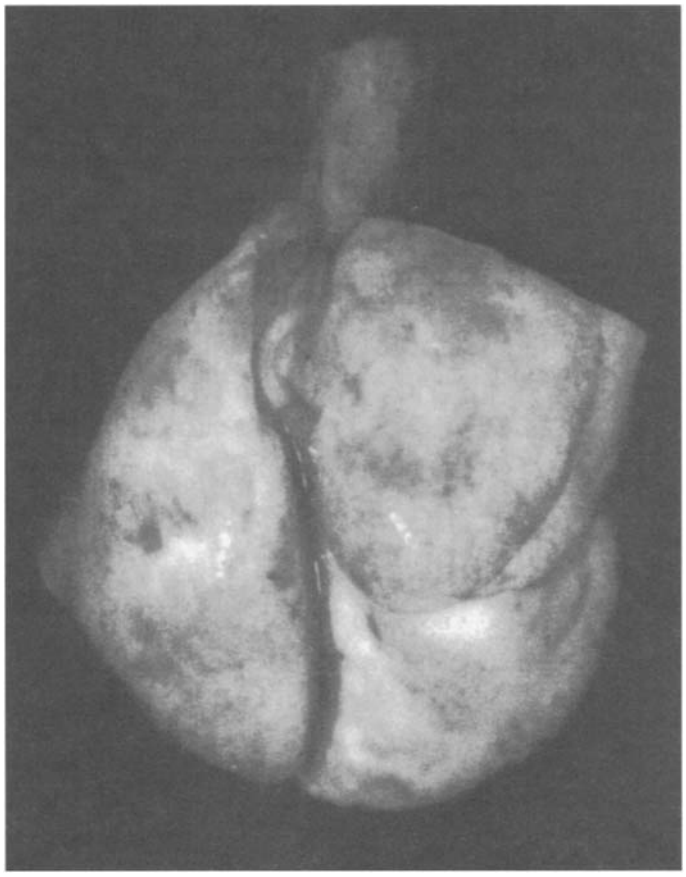

Fig. 44. Lung from a mouse with *Pneumocystis* pneumonia that has failed to collapse after removal.

logical changes are characterized by interstitial alveolitis with thickening of alveolar septa from proteinaceous exudate and infiltration with mononuclear cells (Fig. 45) (Roths *et al.*, 1990). Alveolar spaces may contain vacuolated eosinophilic material and macrophages. Special stains are required to visualize *Pc*. Silver-based stains reveal round or partially flattened 3–5 mm cysts in affected parenchyma (Fig. 46). In florid cases, alveolar spaces may be filled with cysts, but cysts may be sparse in mild cases.

Diagnosis. Respiratory distress in immunodeficient mice should elicit consideration of pneumocystosis. Pathologic examination of the lung, including silver methenamine staining, is essential to confirm a presumptive clinical diagnosis. Past infections of immunocompetent mice also can be detected by ELISA (Furuta *et al.*, 1985). PCR can be used to detect active infection (Gigliotti, *et al.*, 1993; Reddy *et al.*, 1992) and is particularly useful for screening immunodeficient mice.

Differential diagnosis. Pneumocystosis must be differentiated from viral pneumonias of mice. It is worth noting, in this regard,

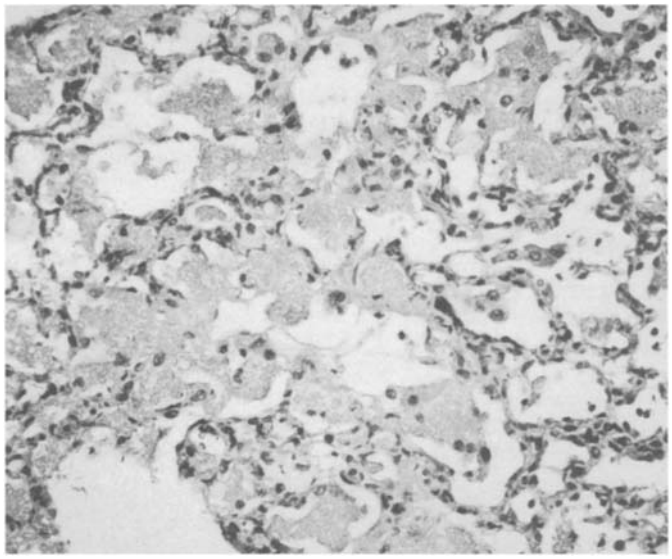

Fig. 45. *Pneumocystis* pneumonia, illustrating hypercellular alveolar septa and alveoli containing proteinaceous exudate and macrophages.

that pneumonia virus of mice has been shown to accelerate the development of pneumocytosis in SCID mice (Bray *et al.*, 1993; Roths *et al.*, 1993).

Prevention and control. *Pc* infection is a significant disease threat to immunodeficient mice. Its widespread distribution strongly suggests that susceptible mice should be protected

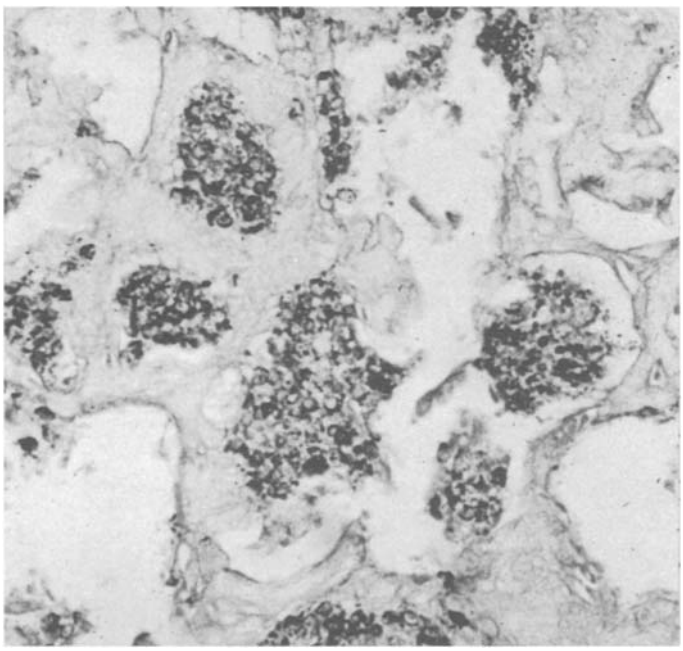

Fig. 46. *Pneumocystis* pneumonia, illustrating *Pneumocystis* cysts in alveoli (Gomori methenamine–silver stain).

by microbarrier combined, where possible, with macrobarrier housing. Husbandry procedures should include proper sterilization of food, water, and housing equipment and the use of HEPA-filtered change stations. Infected colonies can be rederived by embryo transfer or cesarean methods, because infection does not appear to be transmitted *in utero.*

Research complications. Pneumonia in immunodeficient mice is the major complication of *Pc* infection.

b. Dermatomycosis (Ringworm) (Besch-Williford and Wagner, 1982)

Trichophyton mentagrophytes is the most common fungal agent of mice. However, infection rarely causes clinical disease. Clinical signs include sparse hair coats or well-demarcated crusty lesions, with a chalky surface on the head, tail, and legs (favus or ringworm). Skin lesions are composed of exfoliated debris, exudate, mycelia, and arthrospores with underlying dermatitis. Invasion of hair shafts is not characteristic. Diagnosis depends on effective specimen collection. Hairs should be selected from the periphery of the lesion, and hairless skin should be scraped deeply to obtain diagnostic specimens. *Trichophyton mentagrophytes* rarely fluoresces under ultraviolet light, and hyphae must be differentiated from bedding fibers, food particles, and epidermal debris. Histological sections should be stained with a silver stain or Schiff's reagent to reveal organisms. *Trichophyton* also can be cultured on Sabouraud's agar. Plates are incubated at room temperature (22°–30°C), and growth is observed at 5–10 days.

Ringworm is not easily eradicated from laboratory mice. The use of antifungal agents to treat individual mice is time-consuming, expensive, and variably effective. Rederivation is a more prudent course. Cages and equipment should be sterilized before reuse. Concurrent infection with ectoparasites also must be considered during eradication steps.

Candida albicans and other systemic mycoses are not important causes of disease in mice, but they can be opportunistic pathogens in immunodeficient mice.

5. Parasitic Diseases

a. Protozoal Diseases (Hsu, 1982)

i. Giardiasis

Etiology. *Giardia muris* is a pear-shaped, flagellated organism with an anterior sucking disk. It inhabits the duodenum of young and adult mice, rats and, hamsters.

Clinical signs. Infection is often asymptomatic, unless organisms proliferate extensively, and can cause weight loss, a rough hair coat, sluggish movement, and abdominal distension, usually without diarrhea. Additionally, immunodeficient mice may die during heavy infestation.

Epizootiology. The contemporary prevalence of affected mouse colonies is not well documented, but surveys during the 1980s found rates exceeding 50%. Transmission occurs by the fecal–oral route. Cross-infection between mice and hamsters after experimental inoculation of organisms has been demonstrated, whereas rats were resistant to isolates from mice and hamsters (Kunstyr *et al.,* 1992). C3H/He mice are particularly susceptible to giardiasis, whereas BALB/c and C57BL/10 mice are more resistant. Additionally, female mice appear to be more resistant to infection than male mice (Daniels and Belosevic, 1995). C57BL/6 females, for example, have lower trophozoite burdens and for a shorter interval than male mice. Females also shed cysts later than male mice. These differences may be related to a more potent humoral immune response to *Giardia* in female mice.

Pathology. Gross lesions are limited to the small intestine, which may contain yellow or white watery fluid. Histopathology reveals organisms in the lumen that often adhere to microvilli of enterocytes or reside in mucosal crevices or mucus. The crypt–villus ratio may be reduced, and the lamina propria may have elevated numbers of inflammatory cells.

Diagnosis. Diagnosis is based on detection of trophozoites in the small intestine or in wet mounts of fecal material. Organisms can be recognized in wet preparations by their characteristic rolling and tumbling movements. Ellipsoidal cysts with four nuclei also may be detected in feces. Infection also can be detected by serology (Daniels and Belosevic, 1994) and by PCR (Mahbubani *et al.,* 1991).

Treatment, prevention, and control. Murine giardiasis can be treated by the addition of 0.1% dimetridazole to drinking water for 14 days. Prevention and control depend on proper sanitation and management, including adequate disinfection of contaminated rooms.

Research complications. Accelerated cryptal cell turnover and suppression of the immune response to sheep erythrocytes have been observed in infected mice. The potential for severe or lethal infection in immunodeficient mice was noted previously.

ii. Spironucleosis

Etiology. *Spironucleus muris* is an elongated, pear-shaped, bilaterally symmetrical flagellated protozoan that commonly inhabits the duodenum, usually in the crypts of Lieberkühn. It is smaller than *Giardia muris* and lacks an anterior sucking disk.

Clinical signs. *Spironucleus muris* infection is usually asymptomatic in normal adult mice. It is more pathogenic, however, for young, stressed, or immunocompromised mice (Kunstyr *et al.,* 1977). Additionally, clinical morbidity may indicate an underlying primary infection with an unrelated organism.

Clinically affected mice can have a poor hair coat, sluggish behavior, and weight loss. Mice at 3–6 weeks of age are at notably higher risk for clinically evident infection. They can develop dehydration, hunched posture, abdominal distension, and diarrhea. Severe infections can be lethal.

Epizootiology. Transmission occurs by the fecal–oral route and can occur between hamsters and mice as well as between mice. It does not appear to be transmitted between mice and rats (Schagemann *et al.,* 1990). The most recent surveys, which are somewhat dated, indicated that prevalence rates exceeded 60% among domestic mouse colonies in the mid-1980s. There is some evidence that inbred strains vary in their susceptibility to infection and their rate of recovery (Baker *et al.,* 1998; Brett and Cox, 1982).

Pathology. Gross findings associated with infection include watery, red-brown, gaseous intestinal contents. However, it is essential to rule out primary or coinfection by other organisms before attributing such lesions to spironucleosis. Microscopically, acute disease is associated with distension of crypts and intervillous spaces by pear-shaped trophozoites and inflammatory edema of the lamina propria. Organisms can be visualized more easily with periodic acid–Schiff staining, which may reveal invasion of organisms between enterocytes and in the lamina propria. Chronic infection is associated with lymphoplasmacytic infiltration of the lamina propria and perhaps intracryptal inflammatory exudate.

Diagnosis. Diagnosis is based on identification of trophozoites in the intestinal tract. They can be distinguished from *Giardia muris* and *Tritrichomonas muris* by their small size, horizontal or zigzag movements, and the absence of a sucking disk or undulating membrane. PCR-based detection also is available (Rozario *et al.,* 1996). It is not clear whether duodenitis is a primary pathogenic effect of *S. muris* or represents opportunism secondary to a primary bacterial or viral enteritis. Therefore, it is prudent to search for underlying or predisposing infections.

Treatment, prevention, and control. Treatment consists of adding 0.1% dimetridazole to drinking water for 14 days, as described for giardiasis. Prevention and control require good husbandry and sanitation.

Research complications. As with giardiasis, infection can accelerate enterocytic turnover in the small intestine. There is some evidence that infected mice may have activated macrophages that kill tumor cells nonspecifically and that infection can diminish responses to soluble and particulate antigens. Additionally, infected mice also have increased sensitivity to irradiation. Such effects should, however, be interpreted cautiously in order to rule out intercurrent viral infections.

iii. Tritrichomoniasis *Tritrichomonas muris* is a nonpathogenic protozoan that occurs in the cecum, colon, and small intestine of mice, rats, and hamsters. No cysts are formed, and transmission is by ingestion of trophozoites passed in the feces. It can be detected by microscopy or by PCR (Viscogliosi *et al.,* 1993).

iv. Coccidiosis *Eimeria falciformis* is a pathogenic coccidian that occurs in epithelial cells of the large intestines of mice. It was common in European mice historically but is seldom observed in the United States. Heavy infection may cause diarrhea and catarrhal enteritis.

Klosiella muris causes renal coccidioisis in wild mice but is vanishingly rare in laboratory mice. Mice are infected by ingestion of sporulated sporocysts. Sporozoites released from the sporocysts enter the bloodstream and infect endothelial cells lining renal arterioles and glomerular capillaries, where schizogony occurs. Mature schizonts rupture into Bowman's capsule to release merozoites into the lumen of renal tubules. Merozoites can enter epithelial cells lining convoluted tubules, where the sexual phase of the life cycle is completed. Sporocysts form in renal tubular epithelium and eventually rupture host cells and are excreted in the urine, but oocysts are not formed. Infection is usually nonpathogenic and asymptomatic. Gray spots may occur in heavily affected kidneys and are the result of necrosis, granulomatous inflammation, and focal hyperplasia. Destruction of tubular epithelium may impair renal physiology. Diagnosis is based on detection of organisms in tissues. Prevention and control require proper sanitation and management techniques. There is no effective treatment.

v. Cryptosporidiosis *Cryptosporidium muris* is a sporozoan that adheres to the gastric mucosa. It is uncommon in laboratory mice and is only slightly pathogenic. *Cryptosporidium parvum* inhabits the small intestine and is usually nonpathogenic in immunocompetent and athymic mice (Ozkul and Aydin, 1994; Taylor *et al.,* 1999). Athymic mice may develop cholangitis and hepatitis, however, if organisms gain access to the biliary tract.

vi. Entamoebiasis *Entamoeba muris* is found in the cecum and colon of mice, rats, and hamsters throughout the world. Organisms live in the lumen, where they feed on particles of food and bacteria. They are considered nonpathogenic.

vii. Encephalitozoonosis *Encephalitozoon cuniculi* is a gram-positive microsporidian that infects rabbits, mice, rats, guinea pigs, dogs, nonhuman primates, humans, and other mammals. Infection is extremely rare among laboratory mice. The life cycle of the organism is direct, and animals are infected by ingesting spores or by cannibalism. Spore cells are disseminated in the blood to the brain and other sites. Infection can last more than 1 year, and spores shed in the urine serve as a source

of infection. Vertical transmission has not been confirmed in mice. *Encephalitozoon cuniculi* is an obligate intracellular parasite, but infection usually elicits no clinical signs of disease. Organisms proliferate in peritoneal macrophages by asexual binary fission. They have a capsule that accepts Giemsa and Goodpasture stains but is poorly stained by hematoxylin. Fulminating infection can cause lymphocytic meningoencephalitis and focal granulomatous hepatitis. In contrast to encephalitozoonosis in rabbits, affected mice do not develop interstitial nephritis. Infection is diagnosed by cytological examination of ascitic fluid smears, by histopathologic examination of brain tissues stained with Goodpasture stain, and ELISA serology. No effective treatment has been reported. Prevention and control require rigid testing and elimination of infected colonies and cell lines.

viii. Toxoplasmosis *Toxoplasma gondii* is a ubiquitous gram-negative coccidian parasite for which the mouse serves as a principal intermediate host. However, the prevalence of natural infection is negligible because laboratory mice no longer have access to sporulated cysts shed by infected cats, which were historically the major source for cross-infection. Toxoplasmosis can cause necrosis and granulomatous inflammation in the intestine, mesenteric lymph nodes, eyes, heart, adrenals, spleen, brain, lung, liver, placenta, and muscles. Diagnosis is based on ELISA serology and histopathology. Control and prevention depend largely on precluding access of mice to cat feces or to materials contaminated with cat feces. Oocytes are very resistant to adverse temperatures, drying, and chemical disinfectants; therefore thorough cleaning of infected environments is required.

b. Cestodiasis (Wescott, 1982; Potkay, 1994)

Hymenolepis nana (dwarf tapeworm) infestation

Etiology. *Hymenolepis nana,* the dwarf tapeworm, infects mice, rats, and humans. Adults are extremely small (25–40 mm) and have eggs with prominent polar filaments and rostellar hooks (Fig. 47).

Clinical signs. Young adult mice are most frequently infected. Signs and lesions include weight loss and focal enteritis, but clinical disease is rare unless infestation is severe.

Epizootiology. The life cycle may be direct or indirect. (*Hymenolepis nana* is the only cestode known that does not require an intermediate host.) The indirect cycle utilizes arthropods as intermediate hosts. Liberated oncospheres penetrate intestinal villi and develop into a cercocystis stage before reemerging into the intestinal lumen 10–12 days later. The scolex attaches to the intestinal mucosa, where the worm grows to adult size in 2 weeks. The cycle from ingestion to patency takes 20–30 days.

Pathology. Cysticerci are found in the lamina propria of the small intestine and sporadically in the mesenteric lymph nodes, whereas adults, which have a serrated profile, are found in the lumen. Inflammation is not a feature of infection.

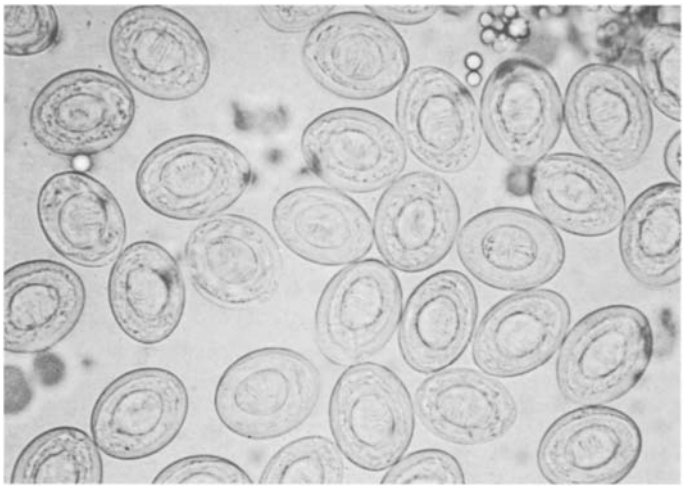

Fig. 47. Eggs of *Hymenolepis nana.*

Diagnosis. Infection can be diagnosed by demonstrating eggs in fecal flotation preparations or by opening the intestine in petri dishes containing warm tap water to facilitate detection of adults. *Hymenolepis nana* can be differentiated from another species of rodent tapeworm, *H. diminuta,* by the fact that *H. nana* has rostellar hooks and eggs with polar filaments. However, *H. diminuta* requires an intermediate arthropod host, so it is rarely found in contemporary mouse colonies.

Treatment, prevention, and control. Drugs recommended for treatment and elimination include praziquantel (0.05% in the diet for 5 days), albendazole, mebendazole, and thiabendazole. Although the benzimidazoles have excellent activity against cestodes and nematodes in rats, they have not been tested extensively in mice. The potential for successful treatment is high, however, because eggs do not survive well outside the host and because the prevalence of infestation is low in caged mice kept in properly sanitized facilities. Because *H. nana* can directly infect humans, proper precautions should be taken to avoid oral contamination during handling of rodents.

Hymenolepis microstoma is found in the bile ducts of rodents and could be confused with *H. nana* in the mouse. However, the location of the adult as well as the large size of *H. microstoma* eggs compared with those of *H. nana* make differential diagnosis relatively simple. The mouse and the rat are intermediate hosts of the cestode *Taenia taeniaformis.* The definitive host is the cat. This parasite should not be found in laboratory mice housed separately from cats.

c. Nematodiasis (Wescott, 1982)

i. Syphacia obvelata (mouse pinworm) infestation

Etiology. *Syphacia obvelata,* the common mouse pinworm, is a ubiquitous parasite of wild and laboratory mice. The rat, gerbil, and hamster are also occasionally infected. Female worms range from 3.4 to 5.8 mm in length, and male worms are smaller

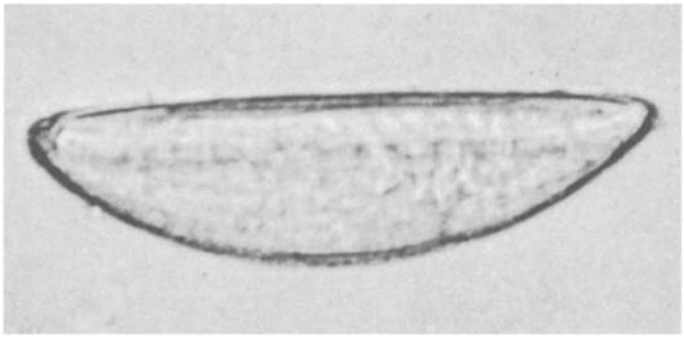

Fig. 48. Syphacia obvelata egg.

(1.1–1.5 mm). Eggs are flattened on one side and have pointed ends (Fig. 48). The nucleus fills the shell and is frequently at a larval stage when eggs are laid.

Clinical signs. Infestation is usually asymptomatic, although heavily infested mice can occasionally sustain intestinal lesions, including rectal prolapse, intussusception, enteritis, and fecal impaction.

Epizootiology. Pinworm infestation is one of the most commonly encountered problems in laboratory mice. A recent national survey revealed that more than 30% of barrier colonies and about 70% of conventional colonies were affected (Jacoby and Lindsey, 1997). The epizootiological impact of pinworm infestation is increased by the airborne dissemination of eggs, which can remain infectious even after drying. The life cycle is direct and completed in 11–15 days. Females deposit their eggs on the skin and hairs of the perianal region. Ingested eggs liberate larvae in the small intestine, and they migrate to the cecum within 24 hr. Worms remain in the cecum for 10–11 days, where they mature and mate. The females then migrate to the large intestine to deposit their eggs as they leave the host. There is unconfirmed speculation that larvae may reenter the rectum. Infestation usually begins in young mice and can recur, but adult mice tend to be more resistant. *Syphacia* infestation often occurs in combination with *Aspicularis tetraptera.* Because the life cycle of *Syphacia* is much shorter than that of *Aspicularis,* the number of mice that are apt to be infected with *S. obvelata* is correspondingly greater. There is evidence that resistance to infestation may be mouse strain-specific (Derothe *et al.,* 1997).

Pathology. Gross lesions are not prevalent, aside from the presence of adults in the lumen of the intestine.

Diagnosis. Infestation is diagnosed by demonstrating reniform-shaped eggs in the perianal area or adult worms in the cecum or large intestine. Four- to 5-week-old mice should be examined because the prevalence is higher in this age group than in older mice. Because most eggs are deposited outside the gastrointestinal tract, fecal examination is not reliable. Eggs are usually detected by pressing cellophane tape to the perineal area and then to a glass slide that is examined by microscopy. *Aspicularis tetraptera* eggs are not ordinarily found in tape preparations and are easily differentiated from eggs of *S. obvelata* (see below). Adult worms can be found in cecal or colonic contents diluted in a petri dish of warm tap water. They are readily observed with the naked eye or with a dissecting microscope. An ELISA also is available to detect serum antibodies to *S. obvelata* somatic antigens (Sato *et al.,* 1995).

Treatment, prevention, and control. Pinworm infestation can be treated effectively by a number of regimens, which include the use of anthelmintics such as piperazine, ivermectin, and benzimidazole compounds alone or in combination (Klement *et al.,* 1996; Le Blanc *et al.,* 1993; Lipman *et al.,* 1994; Flynn *et al.,* 1989; Wescott, 1982; Zenner, 1998). Because some of the recommended therapies have the potential for toxicity, it is prudent to keep mice under close clinical observation during treatment (Davis *et al.,* 1999; Skopets *et al.,* 1996; Toth *et al.,* 2000). Prevention of reinfestation requires strict isolation because *Syphacia* eggs become infective as soon as 6 hr after they are laid, and they survive for weeks even in dry conditions. Strict sanitation, sterilization of feed and bedding, and periodic anthelmintic treatment are required to control infestation. The use of microbarrier cages can reduce the spread of infective eggs.

Research complications. Unthriftiness and perturbation of host immune responses are the primary complications of pinworm infection.

Syphacia muris is the common rat pinworm. It can potentially infest mice but is not found in well-managed colonies. It can be differentiated from *S. obvelata* because *S. muris* eggs are smaller. Treatment is the same as for pinworms of mice.

ii. Aspicularis tetraptera (mouse pinworm) infection

Etiology. Aspicularis tetraptera is the other major oxyurid of the mouse and may coinfest mice carrying *S. obvelata.* Females are 2.6–4.7 mm long, and males are slightly smaller. The eggs are ellipsoidal (Fig. 49).

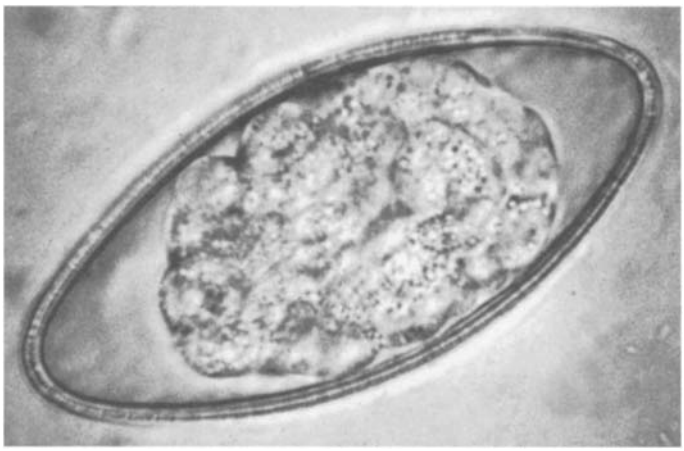

Fig. 49. Aspicularis tetraptera egg.

Clinical signs. Ingested eggs hatch, and larvae reach the middle colon, where they enter crypts and remain for 4–5 days. They move to the proximal colon about 3 weeks after infection of the host. Because the life cycle is 10–12 days longer than in *S. obvelata* (see below), infestations appear in somewhat older mice; heaviest infestation is expected at 5–6 weeks. Infection is usually asymptomatic, but heavy loads can produce signs similar to those discussed for *S. obvelata.* Light to moderate loads do not produce clinical disease.

Epizootiology. As noted under *S. obvelata,* pinworm infestation is highly prevalent and contagious in laboratory mice. The life cycle is direct and takes approximately 23–25 days. Mature females inhabit the large intestine, where they survive from 45 to 50 days and lay their eggs. The eggs are deposited at night and are excreted in a mucous layer, covering fecal pellets. They require 6–7 days at 24°C to become infective and can survive for weeks outside the host.

Pathology. See *S. obvelata* (Section III,A,5,c,i).

Diagnosis. Aspicularis tetraptera eggs can be detected in the feces, and adult worms are found in the large intestine. Eggs are not deposited in the perianal area; therefore cellophane tape techniques are not useful.

Treatment, prevention, and control. Measures for treatment, prevention, and control are similar to those described for *S. obvelata.* Because *A. tetraptera* takes longer to mature and because eggs are deposited in feces rather than on the host, adult parasites are more amenable to treatment by frequent cage rotations. Immune expulsion of parasites and resistance to reinfection are hallmarks of *A. tetraptera* infection.

Research complications. See *S. obvelata* (Section III,A,5,c,i).

d. Acariasis (Mite Infestation) (Weisbroth, 1982)

Several species of mites infest laboratory mice. They include *Myobia musculi, Radfordia affinis, Myocoptes musculinus* and, less commonly, *Psorergates simplex.* The common murine mites are described below, while less frequently encountered insects are shown in Table XIII. These include the mouse mite *Trichoecius romboutsi,* which resembles *Myocoptes* and *Ornithonyssus bacoti,* the tropical rat mite, which can infect laboratory mice. Characteristics of specific infestations are described after a general introductory section.

Clinical signs. Mites generally favor the dorsal anterior regions of the body, particularly the top of the head, neck, and withers (areas least amenable to grooming), but in severe cases, all areas of skin can be infested (Fig. 50). Skin lesions of acariasis include pruritis, scruffiness, patchy hair loss, and, in severe cases, ulceration and pyoderma initiated or compounded by self-inflicted trauma.

Epizootiology. Ectoparasitism in mice is dominated by acariasis. A 1997 survey reported mite infestations in 15% of barrier colonies and 40% of conventional colonies (Jacoby and Lindsey, 1997). Acarids spend their entire lives on the host. Populations

Table XIII

Ectoparasites of Laboratory Mice of the Order Acarina

Suborder	Genus	Species	Common name
Mesostigmata	*Ornithonyssus*	*bacoti*	Tropical rat mite
	Ornithonyssus	*sylviarum*	Northern fowl mite
	Liponyssoides	*sanguineus*	House mouse mite
	Haemogamasus	*pontiger*	
	Eulaelaps	*stabularis*	
	Laelaps	*echidninus*	Spiny rat mite
	Haemolaelaps	*glasgowi*	
	Haemolaelaps	*casalis*	
Prostigmata			
Family Myobiidae			
Subfamily Myobiinae	*Myobia*	*musculi*	Fur mite
	Radfordia	*affinis*	Fur mite
Family Psorergatidae	*Psorergates*	*simplex*	Hair follicle mite
Family Sarcoptidae	*Notoedres*	*musculi*	
Family Demodicidae	*Demodex*	*musculi*	
Astigmata			
Family Myocoptidae	*Myocoptes*	*musculinus*	
	Trichoecius	*romboutsi*	

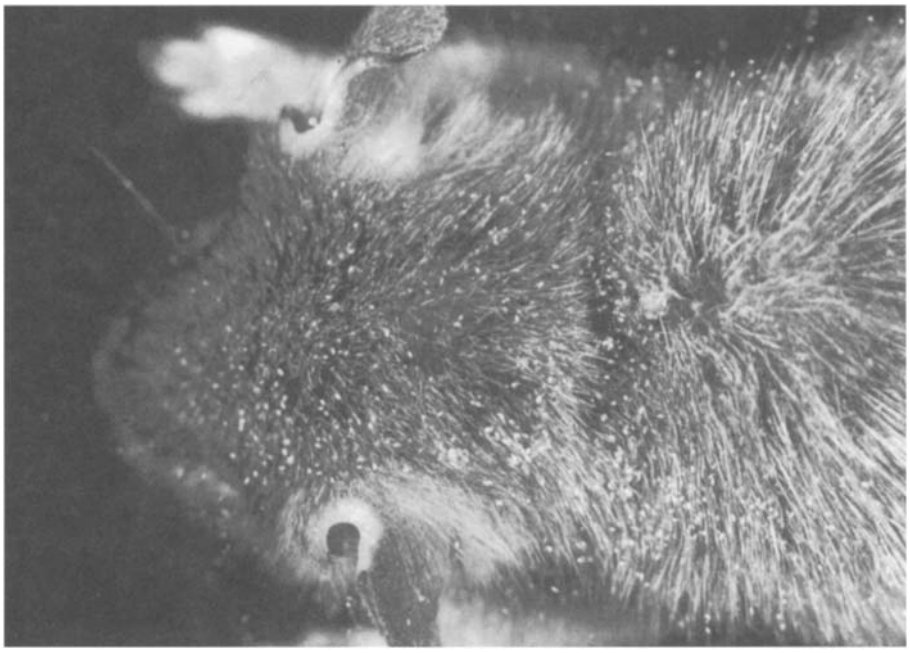

Fig. 50. Acariasis.

are limited by factors such as self-grooming, mutual grooming, the presence of hair, and immunological responses, which tend to produce hypersensitivity dermatitis. Inherited resistance and susceptibility also affect clinical expression of acariasis. Mite populations, for example, vary widely among different stocks and strains of mice housed under similar conditions.

Pathology. Gross lesions include scaly skin, regional hair loss, abrasions, and ulcerations. Histologically, hyperkeratosis, acanthosis, and chronic dermatitis may occur. Long-standing infestation provokes chronic inflammation, fibrosis, and proliferation of granulation tissue. Ulcerative dermatitis associated with acariasis may have an allergic pathogenesis but often results in secondary bacterial infections. Lesions resemble allergic acariasis in other species and are associated with mast cell accumulations.

Diagnosis. Direct observation of the hair and skin of dead or anesthetized mice is simple and straightforward. Hairs are parted with pins or sticks and examined with a dissecting microscope. Examination of young mice, prior to the onset of immune-mediated equilibrium, is likely to be more productive. Alternatively, recently euthanized mice can be placed on a black paper, and double-sided cellophane tape can be used to line the perimeter to contain the parasites. As the carcass cools, parasites will vacate the pelage and crawl onto the paper. Sealed petri dishes can also be used. Cellophane tape also can be pressed against areas of the pelt of freshly euthanatized mice and examined microscopically. Skin scrapings made with a scalpel blade can be macerated in 10% KOH/glycerin or immersion oil and examined microscopically. This method has the disadvantage of missing highly motile species and low-level populations of slower-moving immature forms. It is important to remember that mite infestations may be mixed, so the identification of one species does not rule out the presence of others.

Gross anatomical features facilitate differentiation of intact mites. *Myocoptes* has an oval profile with heavily chitinized body, pigmented third and fourth legs, and tarsal suckers (Fig. 51). *Myobia* and *Radfordia* have a similar elongated profile, with bulges between the legs. *Myobia* has a single tarsal claw on the second pair of legs (Fig. 52), whereas *Radfordia* has two claws of unequal size on the terminal tarsal structure of its second pair of legs (Fig. 53). Histopathological examination of skin is helpful for diagnosing unique forms of acariasis, such as the keratotic cysts associated with *Psorergates simplex* infestation.

Treatment, prevention, and control. Pharmacologic eradication with ivermectin or related compounds is costly, may cause toxic side effects, and is rarely completely effective. Therefore, this approach should be used cautiously. Because of the limitations inherent to currently available treatments, it is preferable to eliminate infestation by gnotobiotic rederivation. Control and prevention programs should be carried out on a colonywide basis, which includes thorough sanitation of housing space and equipment to remove residual eggs.

Research complications. Hypersensitivity dermatitis has the potential to confound immunological studies (Jungmann *et al.*, 1996), especially those involving skin, and has been shown to

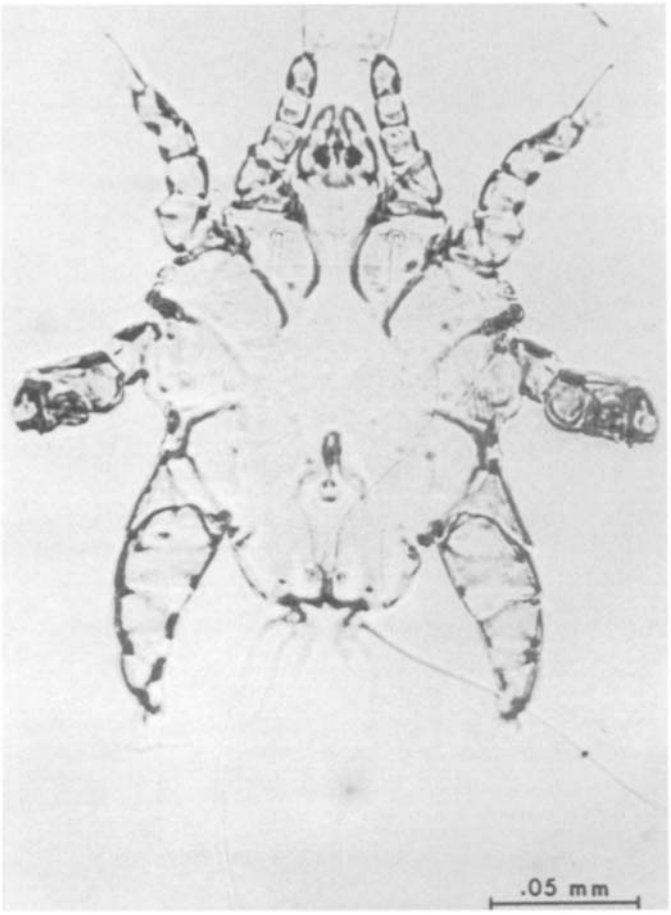

Fig. 51. *Myocoptes musculinus* male. (From Weisbroth, 1982; courtesy of Dr. R. J. Flynn and *Laboratory Animal Science.*)

elevate serum IgE (Morita *et al.,* 1999). Heavy mite infestations can cause severe skin lesions and have been associated with weight loss, infertility, and premature deaths. Chronic acariasis also may provoke secondary amyloidosis due to long-standing dermatitis.

Additional characteristics of murine acariasis

Myocoptes musculinus. This is the most common ectoparasite of the laboratory mouse but frequently occurs in conjunction with *Myobia musculi.* The life cycle includes egg, larva, protonymph, tridonymph, and adult stages. Eggs hatch in 5 days and are usually attached to the middle third of the hair shaft. The life cycle may range from 8 to 14 days. Transmission requires direct contact, for mice separated by wire screens do not contract infestations from infested hosts. Bedding does not seem to serve as a vector. Neonates may become infested within 4–5 days of birth, and parasites may live for 8–9 days on dead hosts.

Myocoptes appears to inhabit larger areas of the body than *Myobia* and tends to crowd out *Myobia* during heavy infestations. It has some predelection for skin of the inguinal region, abdominal skin, and back, but it will also infest the head and neck. It is a surface dweller that feeds on superficial epidermis. Infestation can cause patchy thinning of the hair, alopecia, or erythema. Lesions can be pruritic, but ulceration has not been reported. Chronic infestations induce epidermal hyperplasia and nonsuppurative dermatitis.

Myobia musculi. This is a common mite of laboratory mice. The life cycle of *Myobia* can be completed in 23 days and includes an egg stage, first and second larval stage, protonymph, deutonymph, and adult. Eggs attach at the base of hair shafts and hatch in 7–8 days. Larval forms last about 10 days, followed by nymphal forms on day 11. Adults appear by day 15 and lay eggs within 24 hr.

Myobia are thought to feed on skin secretions and interstitial fluid but not on blood. They are transmitted primarily by contact. Mite populations increase during new infestations, followed by a decrease to equilibrium in 8–10 weeks. The equilibrated population can be carried in colonies for long periods (up to years). Population fluctuations may represent waves of egg

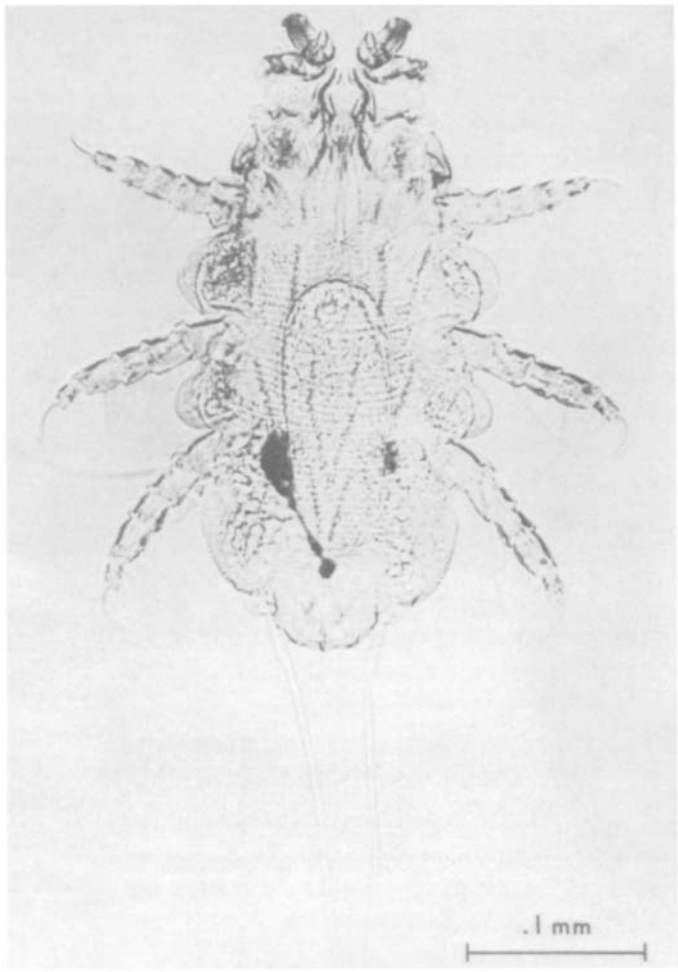

Fig. 52. *Myobia musculi* female. (From Weisbroth, 1982; courtesy of Dr. R. J. Flynn and *Laboratory Animal Science.*)

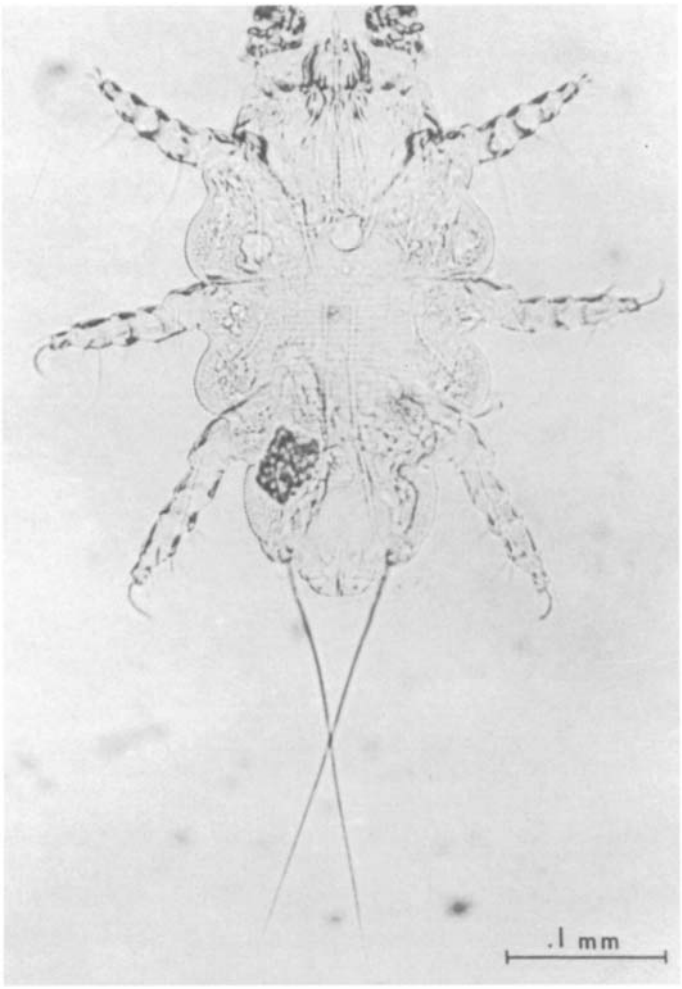

Fig. 53. *Radfordia affiinis* female. (From Weisbroth, 1982; courtesy of Dr. R. J. Flynn and *Laboratory Animal Science.*)

hatchings. Because mites are thermotactic, they crawl to the end of hair shafts on dead hosts, where they may live for up to 4 days. Infestation may result in hypersensitivity dermatitis, to which C57BL mice are highly susceptible. Clinical signs vary from ruffled fur and alopecia to pruritic ulcerative dermatitis. Therefore, lesions can be exacerbated by self-inflicted trauma.

Radfordia affinis. *Radfordia* is thought to be common in laboratory mice, but it closely resembles *Myobia* and may occur as a mixed infestation. Therefore, its true prevalence is conjectural. Additionally, its life cycle has not been described. It does not appear to cause clinical morbidity.

Psorergates simplex. This species has not been reported as a naturally occurring infection in well-managed colonies for several decades, but it is unique in that it inhabits hair follicles. Its life cycle is unknown, but developmental stages from egg to adult may be found in a single dermal nodule. Transmission is by direct contact. Invasion of hair follicles leads to development of cystlike nodules, which appear as small white nodules in the subcutis. Histologically, they are invaginated sacs of squamous epithelium, excretory products, and keratinaceous debris. There is usually no inflammatory reaction, but healing may be accompanied by granulomatous inflammation. Diagnosis is made by examining the subcuticular surface of the pelt grossly or by histological examination. Sac contents also can be expressed by pressure with a scalpel blade or scraped and mounted for microscopic examination.

B. Metabolic and Nutritional Diseases

1. Amyloidosis

Amyloidosis is caused by the deposition of insoluble (polymerized) proteins and occurs in primary and secondary forms. Primary amyloidosis is a naturally occurring disease in mice, associated with the deposition of amyloid proteins consisting primarily of immunoglobulin light chains. Secondary amyloidosis is associated with antecedent and often chronic inflammation. It results from a complex cascade of reactions involving release of multiple cytokines that stimulate amyloid synthesis in the liver (Falk and Skinner, 2000).

Primary amyloidosis is common among aging mice (Lipman *et al.,* 1993) but also may occur in young mice of highly susceptible strains such as A and SJL or somewhat later among C57BL mice. Other strains, such as BALB/c and C3H are highly resistant to amyloidosis (Dunn, 1967). Secondary amyloidosis is usually associated with chronic inflammatory lesions, including dermatitis resulting from prolonged acariasis. It can be induced experimentally, however, by injection of casein and may occur locally in association with neoplasia or in ovarian corpora lutea in the absence of other disease. Amyloidosis can shorten the life span of mice and can be accelerated by stress from intercurrent disease.

Amyloid appears as interstitial deposition of a lightly eosinophilic, acellular material in tissues stained with hematoxylin and eosin. However, it is birefringent after staining with Congo red when viewed with polarized light. Deposition patterns vary with mouse strain and amyloid type. Although virtually any tissue may be affected, the following sites are common: hepatic portal triads, periarteriolar lymphoid sheaths in spleen, renal glomeruli and interstitium (which can lead to papillary necrosis), intestinal lamina propria, myocardium (and in association with atrial thrombosis), nasal submucosa, pulmonary alveolar septa, gonads, endocrine tissues, and great vessels (Fig. 54).

2. Soft Tissue Mineralization

Naturally occurring mineralization of the myocardium and epicardium and other soft tissues is a common finding at

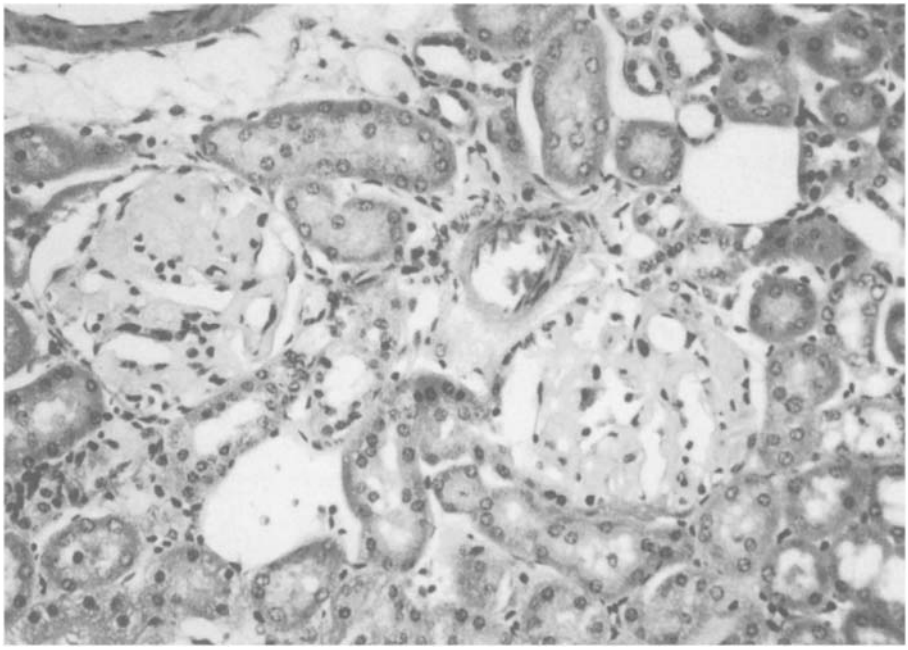

Fig. 54. Renal amyloidosis with prominent amyloid deposition in glomeruli.

necropsy in some inbred strains of mice. It occurs in BALB/c, C3H, and especially DBA mice (Eaton *et al.,* 1978; Brownstein, 1983; Brunnert *et al.,* 1999). It is found in the myocardium of the left ventricle (Fig. 55), in the intraventricular systems, and in skeletal muscle, kidneys, arteries, and lung and may be accompanied by fibrosis and mononuclear inflammatory infiltrates. DBA mice also can develop mineralization in the tongue and cornea. Dietary, environmental, disease-related, and endocrine-related factors are thought to influence the prevalence of this lesion. Although this condition is usually an incidental finding at necropsy, interference with myocardial function cannot be ruled out if lesions are severe.

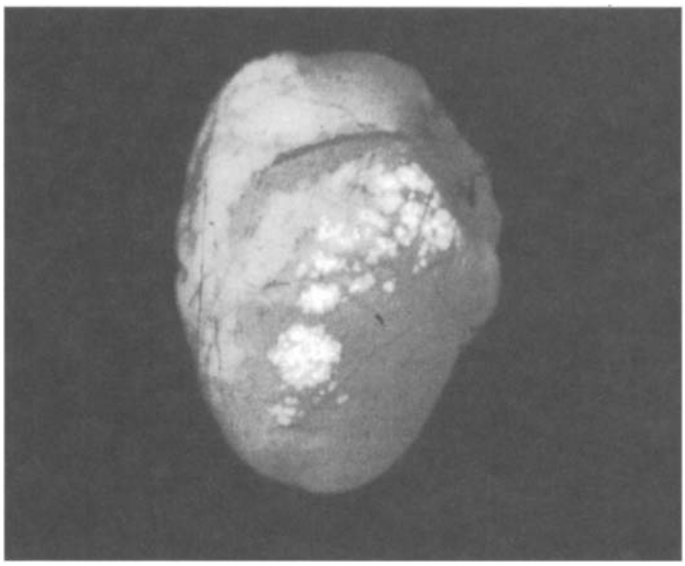

Fig. 55. Epicardial mineralization.

3. Reye's-like Syndrome

A Reye's-like syndrome has been reported in BALB/cByJ mice (Brownstein *et al.,* 1984). The etiology is unknown; however, antecedent viral infection may be involved. Affected mice rapidly become lethargic and then comatose. They also tend to hyperventilate. High mortality ensues within 6–18 hr, but some mice may recover. Lesions are characterized grossly by swollen, pale liver and kidneys. The major histopathological findings include swollen hepatocytes with fatty change and nuclear swelling among astrocytes in the brain. Hepatic lesions resembling changes in Reye's syndrome have been reported in SCID mice infected with MAdV-1 (Pirofski *et al.,* 1991).

4. Vitamin, Mineral, and Essential Fatty Acid Deficiencies (Knapka, 1983)

Vitamin deficiencies in mice have not been thoroughly described. Unfortunately, much of the information that does exist reflects work done 30–50 years ago; thus the reliability and specificity of some of these syndromes is questionable. Vitamin A deficiency may produce tremors, diarrhea, rough hair coat, keratitis, poor growth, abscesses, hemorrhages, and sterility or abortion. Vitamin E deficiency can cause convulsions and heart failure, as well as muscular dystrophy and hyaline degeneration of muscles. Deficiency of B complex vitamins produces nonspecific signs such as alopecia, decreased feed consumption,

poor growth, poor reproduction and lactation, as well as a variety of neurological abnormalities. Choline deficiency produces fatty livers and nodular hepatic hyperplasia, as well as myocardial lesions, decreased conception, and decreased viability of litters. Folic acid–deficient diets cause marked decreases in red and white cell blood counts and the disappearance of megakaryocytes and nucleated cells from the spleen. Pantothenic acid deficiency is characterized by nonspecific signs, such as weight loss, alopecia, achromotrichia, and posterior paralysis, as well as other neurological abnormalities. Thiamin deficiency is associated with neurological signs, such as violent convulsions, cartwheel movements, and decreased food consumption. Dietary requirements for ascorbic acid have not been shown in mice, and mouse diets are generally not fortified with ascorbic acid.

Mineral deficiencies have been described only for several elements, and the consequences of the deficiencies are similar to those observed for other species. For example, iodine-deficient diets produce thyroid goiters; magnesium-deficient diets may cause fatal convulsions; manganese deficiency may cause congenital ataxia from abnormal development of the inner ear; and zinc deficiency may cause hair loss on the shoulders and neck, emaciation, decreased liver and kidney catalase activity, and immunosuppression.

Chronic essential fatty acid deficiency may cause hair loss, dermatitis with scaling and crusting of the skin, and occasional diarrhea. Infertility has also been associated with this syndrome. Mice have an absolute requirement for a dietary source of linoleic and/or arachadonic acid.

5. Alopecia and Chronic Ulcerative Dermatitis in Black Mice (Sundberg, 1994; Ward *et al.*, 2000)

Black mice are prone to a skin condition characterized by alopecia and/or chronic ulcerative dermatitis. It is most often seen among C57BL/6 and C57BL/10 mice because they are used widely. Initial signs include alopecia and papular dermatitis, which usually occur over the dorsal trunk (Fig. 56). In severe cases, skin ulceration ensues, which is grounds for euthanasia, while scarring and disfigurement can occur among surviving mice. The cause is unknown. Microbiological assessments have yielded a variety of bacteria that are considered to be secondary opportunists, and acariasis has not been incriminated. Seasonal fluctuation in the incidence of disease suggests that environmental factors may play a role. The incidence appears to increase during periods of significant seasonal changes in temperature and humidity, i.e., the onset of winter and early spring. There is some evidence that incidence is related to diet, with mice on ad libitum diets being more susceptible than those on restricted diets. However, specific dietary factors have not been identified.

6. Postpartum Ileus

Ileus associated with high mortality has been reported to occur in primiparous female mice during the second week of lactation (Kunstyr, 1986). The cause is unknown.

C. Environmental, Behavioral, and Traumatic Disorders

Environmental variables can affect responses of mice in experimental situations. Changes in respiratory epithelial physiology and function from elevated levels of ammonia, effects of temperature and humidity on metabolism, effects of light on eye lesions and retinal function, and effects of noise on neurophysiology are examples of complications that can vary with the form of insult and the strain of mouse employed.

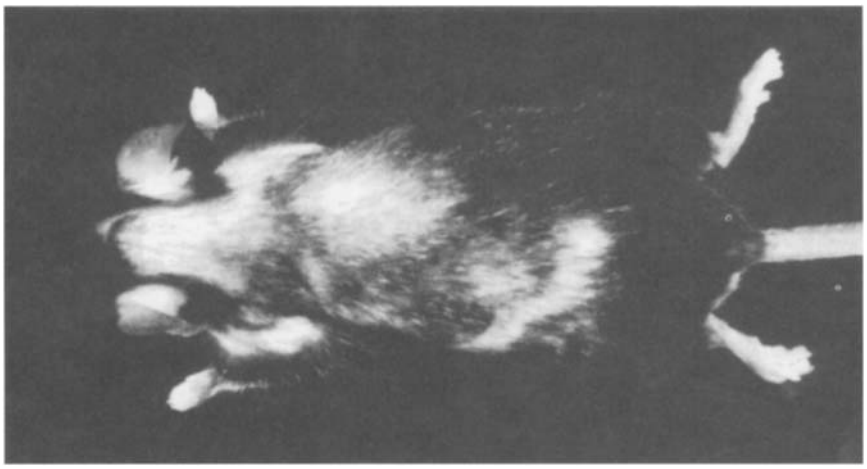

Fig. 56. Dorsal alopecia in a C57BL/6 mouse during an early stage of chronic uclerative dermatitis.

1. Temperature-Related Disorders

Mice do not easily acclimatize to sudden and dramatic changes in temperature. Therefore they are susceptible to both hypothermia and hyperthermia. Mice also are susceptible to dehydration. Poorly functioning water bottles, resulting in spills (hypothermia) or obstructed sipper tubes (dehydration), are probably the major cause of these environmental insults.

2. Ringtail

Ringtail is a condition associated with low relative humidity. Clinical signs include annular constriction of the tail and occasionally of the feet or digits, resulting in localized edema that can progress to dry gangrene (Fig. 57). It should be differentiated from dryness and gangrene that may occur in hairless mice exposed to low temperatures and perhaps other environmental or nutritional imbalances. Necrosis of legs, feet, or digits also can occur in suckling mice because of disruption of circulation by wraps of stringy nesting material such as cotton wool.

3. Corneal Opacities

Corneal opacities can occur as a result of acute or chronic keratitis. There is some evidence that the buildup of ammonia in mouse cages may contribute to this condition, because it can be controlled by increasing the frequency of cage cleaning.

4. Malocclusion

Malocclusion results from an inherited trait for poorly aligned incisors and results in overgrowth, especially of the lower incisors. Abscesses or necrosis may occur in the lips or oral cavity because of fighting injury or mechanical trauma from cages. Overgrown incisor teeth can cause malocclusion, and caries can develop in molar teeth.

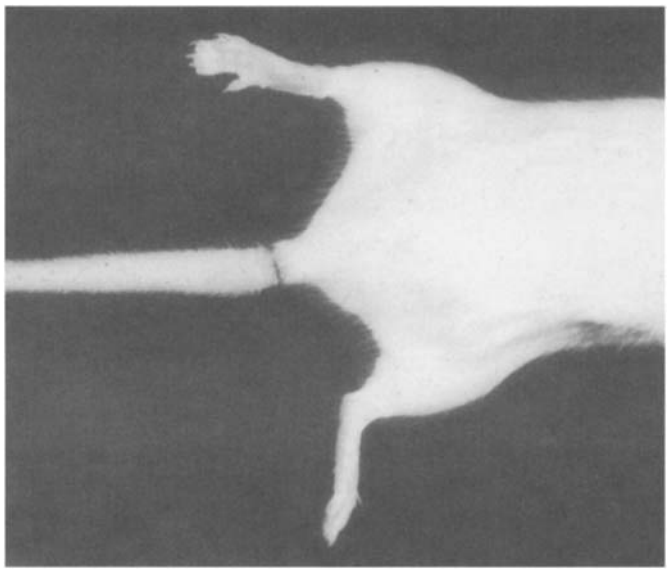

Fig. 57. Ringtail.

5. Skin Trauma

Skin lesions can be caused by fighting, tail biting, and whisker chewing. Fighting is not limited extensively to males, but they tend to be more aggressive. Bite wounds are usually located on the head, neck, shoulders, peritoneal area, and tail. Often one animal per pen is free of lesions and is considered the aggressor or dominant animal. Removal of the unaffected male usually ends the fighting, and the wounded animals recover. However, a previously submissive male may become dominant when an aggressive male is removed, and fighting may resume. Fighting has some strain predilection, and is especially notorious among BALB/c males, but tail lesions resembling bite wounds have been reported in other strains.

Hair nibbling or whisker chewing (barbering) is also a manifestation of social dominance. Dominant animals retain whiskers, whereas cagemates have "shaved faces" (Fig. 11). Chronic hair chewing can produce histological abnormalities such as poorly formed or pigmented club hairs. Once chewing has ceased, many mice regrow previously lost hair in several weeks. Both sexes may engage in this activity, and sometimes females may be dominant. Regional alopecia, especially around the muzzle, may result from abrasion against cage surfaces. Improperly diluted disinfectants may also cause regional hair loss. Metal tags used for animal identification may cause pruritis and self-induced trauma. Clipping prior to application of experimental compounds to the skin may cause pruritic responses and can augment lesions that interfere with test results. Dermatophytosis, ectoparasitism, or idiopathic hair loss must be considered in the differential diagnoses for muzzle or body alopecia.

D. Congenital, Aging-Related, and Miscellaneous Disorders (Burek *et al.* 1982; Percy and Barthold, 2001f)

1. Cardiovascular System

Atrial thrombosis appears to be strain-related, with a high prevalence in RFM mice. It also is more common in aged mice. It usually involves the left atrium and auricle and may be accompanied by amyloidosis. Affected mice may display signs of heart failure, particularly severe dyspnea. *Myocardial and epicardial mineralization* is described above, in Section III,B,2. Periarteritis occurs in aged mice.

2. Respiratory Tract

Hyperplasia of alveolar or bronchial epithelium occurs in old mice and must be differentiated from pulmonary tumors. *Pulmonary histiocytosis* is an incidental finding in selected strains of mice, including C57BL/6, and the incidence increases with

aging. Histologically, alveoli are filled with macrophages containing eosinophilic crystalline material.

3. Alimentary Tract

a. Stomach

Gastric lesions include crypt dilatation, submucosal fibrosis, adenomatous gastric hyperplasia, mineralization, and erosion or ulceration. Gastric ulcers may be stress-related, especially in mice with prolonged illness. Germfree mice may have reduced muscle tone in the intestinal tract. Cecal volvulus is a common finding in germfree mice and is caused by rotation of the large cecum.

b. Liver

Age-associated lesions are common in the livers of mice. Cellular and nuclear pleomorphism, including binucleated and multinucleated cells, are detectable by 6 months. Mild focal necrosis occurs with or without inflammation, but an association of mild focal hepatitis with a specific infectious disease is often hard to confirm. Other geriatric hepatic lesions include biliary hyperplasia with varying degrees of portal hepatitis, hepatocellular vacuolization, amyloid deposition (especially in periportal areas), strangulated or herniated lobes, hemosiderosis, lipofuscinosis, and fibrosis. Extramedullary hematopoiesis occurs in young mice and in response to anemia.

c. Pancreas

Exocrine pancreatic insufficiency has been reported in CBA/J mice. Acinar cell atrophy is common but is strain- and sex-dependent.

4. Lymphoreticular System

Blood-filled mesenteric lymph nodes may occur in aged mice, especially C3H mice. This condition is an incidental finding and should not be confused with infectious lymphadenopathy such as that associated with salmonellosis. Aggregates, or nodules of mononuclear cells, are found in many tissues of aged mice, including the salivary gland, thymus, ovary, uterus, mesentery and mediastinum, urinary bladder, and gastrointestinal tract. These nodules should not be mistaken for lymphosarcomas. The spleen is subject to amyloidosis and hemosiderin deposition. Lipofuscin deposition is common, especially in older mice. The thymus undergoes age-associated atrophy.

A variety of genetic immunodeficiencies have been described in mice, many of which increase susceptibility to infectious diseases. Perhaps the most widely known of these is the athymic nude mouse that lacks a significant hair coat and, more important, fails to develop a thymus and thus has a severe deficit of T cell–mediated immune function. Additionally, severe combined immunodeficient (SCID) mice, which lack both T and B lymphocytes, are used widely used and are highly susceptible to opportunistic agents such as *Pneumocystis carinii.* Specific immune deficits have become excellent models for studying the ontogeny and mechanisms of immune responsiveness (Table XII).

5. Musculoskeletal System

Age-associated osteoporosis or senile osteodystrophy can occur in some mice. It is not associated with severe renal disease or parathyroid hyperplasia. Nearly all strains of mice develop some form of osteoarthrosis. It is generally noninflammatory, affects articulating surfaces, and results in secondary bone degeneration.

6. Urinary Tract

Glomerulonephritis is a common kidney lesion of mice. It is more often associated with persistent viral infections or immune disorders rather than with bacterial infections. Its prevalence in some strains approaches 100%. NZB and NZB × NZW F_1 hybrid mice, for example, develop immune complex glomerulonephritis as an autoimmune disease resembling human lupus erythematosus, whereas glomerular disease is relatively mild in NZB mice (NZB mice have a high incidence of autoimmune hemolytic anemia). Renal changes occur as early as 4 months of age, but clinical signs and severe disease are not present until 6–9 months. The disease is associated with wasting and proteinurea, and lesions progress until death intervenes. Histologically, glomeruli have proteinaceous deposits in the capillaries and mesangium. Later, tubular atrophy and proteinaceous casts occur throughout the kidney. Immunofluorescence studies show deposits of immunoglobulin and the third component of complement, which lodge as immune complexes with nuclear antigens and antigens of murine leukemia virus in glomerular capillary loops. Mice infected with LCMV or with retroviruses can also develop immune complex glomerulonephritis.

Mice also can develop chronic glomerulopathy characterized by progressive thickening of glomerular basement membrane by PAS-positive material that does not stain for amyloid. This lesion can be accompanied by proliferation of mesangial cells; local, regional, or diffuse mononuclear cell infiltration; and fibrosis. Advanced cases may lead to renal insufficiency or failure.

Interstitial nephritis can be caused by bacterial or viral infections but may also be idiopathic. Typical lesions include focal, regional, or diffuse interstitial infiltration of tubular parenchyma by mononuclear cells, but glomerular regions also may be involved. Severe lesions can be accompanied by fibrosis, distortion of renal parenchyma, and intratubular casts, but not by mineralization. If renal insufficiency or failure ensues, it can lead to ascites.

Some strains of mice, such as BALB/c, can develop polycystic kidney disease, which, if severe, can compromise normal renal function.

Urinary tract obstruction occurs as an acute or chronic condition in male mice. Clinical signs usually include wetting of the perineum from incontinence. In severe or chronic cases, wetting predisposes to cellulitis and ulceration. At necropsy, the bladder is distended, and proteinaceous plugs are often found in the neck of the bladder and proximal urethra. In chronic cases the urine may be cloudy, and calculi may develop in the bladder. Additionally, cystitis, urethritis, prostatitis, balanoposthitis, and hydronephrosis may develop. This condition must be differentiated from infectious cystitis or pyelonephritis and from the agonal release of secretions from accessory sex glands, which is not associated with an inflammatory response. Hydronephrosis per se also may occur without urinary tract obstruction.

Ascending pyelitis occurs in mice secondary to urinary tract infection.

7. Genital Tract

a. Female

Parvovarian cysts are observed frequently and may be related to the fact that mouse ovaries are enclosed in membranous pouches. Amyloidosis is also common in the ovaries of old mice. Cystic endometrial hyperplasia may develop unilaterally or bilaterally and may be segmental. In some strains, the prevalence in mice older than 18 months is 100%. Endometrial hyperplasia is often associated with ovarian atrophy. Mucometra is relatively common in adult female mice. The primary clinical sign is abdominal distension resembling pregnancy among mice that do not whelp.

b. Male

Testicular atrophy, sperm granulomas, and tubular mineralization occur with varying incidence. Inflammation of accessory sex glands may occur. Preputial glands, especially of immunodeficient mice, can become infected with opportunistic or pathogenic bacteria.

8. Endocrine System

Accessory adrenal cortical nodules are found in periadrenal and perirenal fat, especially in females. These nodules have little functional significance other than their potential effect on failures of surgical adrenalectomy. Lipofuscinosis, subcapsular spindle cell hyperplasia, and cystic dilatation of cortical sinusoids are found in the adrenal cortices of aged mice.

Some inbred strains have deficiencies of thyrotropic hormone, resulting in thyroid atrophy. Thyroid cysts lined by stratified squamous epithelium and generally of ultimobranchial origin may be seen in old mice. Amyloid can be deposited in the thyroid and parathyroid glands as well as in the adrenal glands. Spontaneous diabetes mellitius occurs in genetic variants of several strains such as nonobese diabetic (NOD) mice.

High levels of estrogen in pregnancy may influence postpartum hair shedding. Various endocrine effects on hair growth have also been described. Abdominal and thoracic alopecia have been reported in B6C3F_1 mice.

9. Nervous System

Symmetrical mineral deposits commonly occur in the thalamus of aged mice. They may also be found in the midbrain, cerebellum, and cerebrum and are particularly common in A/J mice. Lipofuscin accumulates in the neurons of old mice. Age-associated peripheral neuropathy with demyelination can be found in the nerves of the hindlimbs in C57BL/6 mice. Deposits of melanin pigment occur in heavily pigmented strains, especially in the frontal lobe. A number of neurologically mutant mice have been described. They commonly have correlative anatomical malformations or inborn errors of metabolism.

10. Organs of Special Sense

a. Eye

Retinal degeneration can occur as either an environmental or a genetic disorder in mice. Nonpigmented mice, both inbred and outbred, can develop retinal degeneration from exposure to light, with the progression of blindness being related to light intensity and duration of exposure. Other strains such as C3H and CBA are genetically predisposed to retinal degeneration. C3H/He mice express the *rd* gene, which leads to retinal degeneration within the first few weeks of life and has been used extensively as a model for retinitis pigmentosa (Farber and Danciger, 1994). Blindness does not interfere with health or reproduction and blind mice cannot be distinguished from non-blind mice housed in standard caging. Cataracts can occur in old mice and have a higher prevalence in certain mutant strains.

b. Ear

Vestibular syndrome associated with head tilt, circling or imbalance can result from infectious otitis or from necrotizing vasculitis of unknown etiology affecting small and medium-sized arteries in the vicinity of the middle and inner ear.

E. Neoplastic Diseases (Jones *et al.*, 1983–1993; Maronpot *et al.*, 1999; Percy and Barthold, 2001g)

1. Lymphoreticular and Hematopoietic Systems

Neoplasms of lymphoid and hematopoietic tissues are estimated to have a spontaneous prevalence of 1–2%. There are,

however, some strains of mice that have been specifically inbred and selected for susceptibility to spontaneous tumors. Leukemogenesis in mice may involve viruses and chemical or physical agents. Viruses associated with lymphopoietic and hematopoietic neoplasia belong to the family Retroviridae (type C oncornaviruses) and contain RNA-dependent DNA polymerase (reverse transcriptase). These viruses are generally noncytopathogenic for infected cells, and mice appear to harbor them as normal components of their genetic apparatus. Although they may be involved in spontaneous leukemia, they are not consistently expressed in this disease. Recombinant viruses have recently been discovered that can infect mouse cells and heterologous cells and are associated with spontaneous leukemia development in high leukemia strains such as AKR mice. Their phenotypic expression is controlled by mouse genotype. Endogenous retroviruses are transmitted vertically through the germ line. Horizontal transmission is inefficient but can occur by interuterine infection or through saliva, sputum, urine, feces, or milk. The leukemia induced by a given endogeous virus is usually of a single histopathological type. Chemical carcinogens, such as polycyclic hydrocarbons, nitrosoureas, and nitrosamines and physical agents such as X-irradiation can also induce hematological malignancies in mice.

a. Lymphoblastic Lymphoma (Thymic Lymphoma, B-Cell Lymphoma)

The most common hematopoietic malignancy in the mouse is lymphocytic leukemia that originates in the thymus. Disease begins with unilateral atrophy and then enlargement of one lobe of thymus as tumor cells proliferate. Cells can spread to the other lobe and then to other hematopoietic organs, such as the spleen, bone marrow, liver, and peripheral lymph nodes. Clinical signs include dyspnea and ocular protrusion. The latter sign is due to compression of venous blood returning from the head. Tumor cells spill into the circulation late in disease. Most of these tumors originate from T lymphocytes or lymphocblasts, but there are leukemias of B lymphocyte or null cell lineage. In the last two syndromes, the lymph nodes and spleen are often involved, but the thymus is generally normal.

b. Reticulum Cell Sarcoma (Histiocytic Lymphoma, Follicular Center Cell Lymphoma)

Reticulum cell sarcomas are common in older mice, especially in inbred strains such as C57BL/6 and SJL. Primary tumor cell types have been divided into several categories based on morphological and immunohistochemical features. Histiocytic sarcomas correspond to the older Dunn classification as type A sarcomas and are composed primarily of reticulum cells. The tumor typically causes splenomegaly and nodular lesions in other organs, including liver, lung, kidney, and the female reproductive tract. Follicular center cell lymphomas correspond to Dunn type B sarcomas. They originate from B cell regions (germinal centers) of peripheral lymphoid tissues, including spleen, lymph nodes, and Peyer's patches. Typical tumor cells have large vesiculated, folded, or cleaved nuclei and ill-defined cytoplasmic borders. Tumors also often contain small lymphocytes. Type C reticulum cell tumors often involve one or several lymph nodes rather than assuming a wide distribution. They consist of reticulum cells with a prominent component of well-differentiated lymphocytes.

c. Myelogenous Leukemia

Myelogenous leukemia is uncommon in mice and is associated with retrovirus infection. Disease begins in the spleen, resulting in marked splenomegaly, but leukemic spread results in involvement of many tissues including liver, lung, and bone marrow. Leukemic cells in various stages of differentiation can be found in peripheral blood. In older animals, affected organs may appear green because of myeloperoxidase activity, giving rise to the term chloroleukemia. The green hue fades on contact with air. Affected mice are often clinically anemic and dyspneic.

d. Erythroleukemia

Erythroleukemia is rare in mice. The major lesion is massive splenomegaly, which is accompanied by anemia and polycythemia. Hepatomegaly can follow, but there is little change in the thymus or lymph nodes.

e. Mast Cell Tumors

Mast cell tumors are also very rare in mice. They are found almost exclusively in old mice and grow slowly. They should not be confused with mast cell hyperplasia observed in the skin following painting with carcinogens or X-irradiation.

f. Plasma Cell Tumors

Natural plasma cell tumors are infrequent in the mouse. They can, however, be induced by intraperitoneal inoculation of granulomatogenic agents such as plastic filters, plastic shavings, or a variety of oils.

2. Mammary Gland

Mammary tumors can be induced or modulated by a variety of factors, including viruses, chemical carcinogens, radiation, hormones, genetic background, diet, and immune status. Certain inbred strains of mice, such as C3H, A, and DBA/2, have a high natural prevalence of mammary tumors. Other strains, such as BALB/c, C57BL, and AKR, have a low prevalence.

Among the most important factors contributing to the development of mammary tumors are mammary tumor viruses. Several major variants are known. The primary tumor virus

MMTV-S (Bittner virus) is highly oncogenic and is transmitted through the milk of nursing females. Infected mice typically develop a precursor lesion, the hyperplastic alveolar nodule, which can be serially transplanted.

Spontaneous mammary tumors metastasize with high frequency, but this property is somewhat mouse strain dependent. Metastases go primarily to the lung. Some mammary tumors are hormone dependent, some are ovary dependent, and others are pregnancy dependent. Ovary-dependent tumors contain estrogen and progesterone receptors, whereas pregnancy-dependent tumors have prolactin receptors. Ovariectomy will dramatically reduce the incidence of mammary tumors in C3H mice. If surgery is done in adult mice 2–5 months of age, mammary tumors will develop, but at a later age than normal.

Grossly, mammary tumors may occur anywhere in the mammary chain. They present as one or more firm, well-delineated masses, which are often lobular and maybe cystic (Fig. 58). Histologically, mammary tumors have been categorized into three major groups; carcinomas, carcinomas with squamous cell differentiation, and carcinosarcomas. The carcinomas are divided into adenocarinoma types A, B, C, Y, L, and P. Most tumors are type A or B. Type A consists of adenomas, tubular carcinomas, and alveolar carcinomas. Type B tumors have a variable pattern with both well-differentiated and poorly differentiated regions. They may consist of regular cords or sheets of cells or papillomatous areas. These two types are locally invasive and may metastasize to the lungs. Type C tumors are rare and are characterized by multiple cysts lined by low cuboidal to squamous epithelial cells, and they have abundant stroma. Type Y tumors, which are also rare, are characterized by tubular branching of cuboidal epithelium and abundant stroma. Adenocarcinomas with a lacelike morphology (types L and P) are hormone dependent and have a branching tubular structure.

The control or prevention of mammary neoplasms depends on the fact that some strains of mammary tumor virus are transmitted horizontally whereas others are transmitted vertically. Although one can rid mice of horizontally transmitted virus such as MMTV-S by cesarean rederivation or by foster nursing, endogenous strains of tumor virus may remain. Fortunately, these latter tumor viruses have generally low oncogenicity relative to the Bittner virus.

3. Liver

Mice develop an assortment of liver changes as they age, including proliferative lesions. The latter can range from hyperplastic foci to hepatomas to hepatocellular carcinomas. Almost all strains of mice have a significant prevalence of hepatic tumors, some of which appear to result from dietary contamination or deficiency. The prevalence of spontaneous liver tumors in $B6C3F_1$ hybrids is increased by feeding choline-deficient diets. Tumors also can develop in mice exposed to environmental chemicals, many of which are carcinogenic or potentially carcinogenic.

Spontaneous liver tumors in mice occur grossly as gray to tan nodules or large, poorly demarcated dark red masses. They are usually derived from hepatocytes, whereas cholangiocellular tumors are rare. Hepatomas are well circumscribed and well differentiated, but they compress adjacent liver tissue as they develop. Hepatocellular carcinomas are usually invasive and display histopathological patterns ranging from medullary to

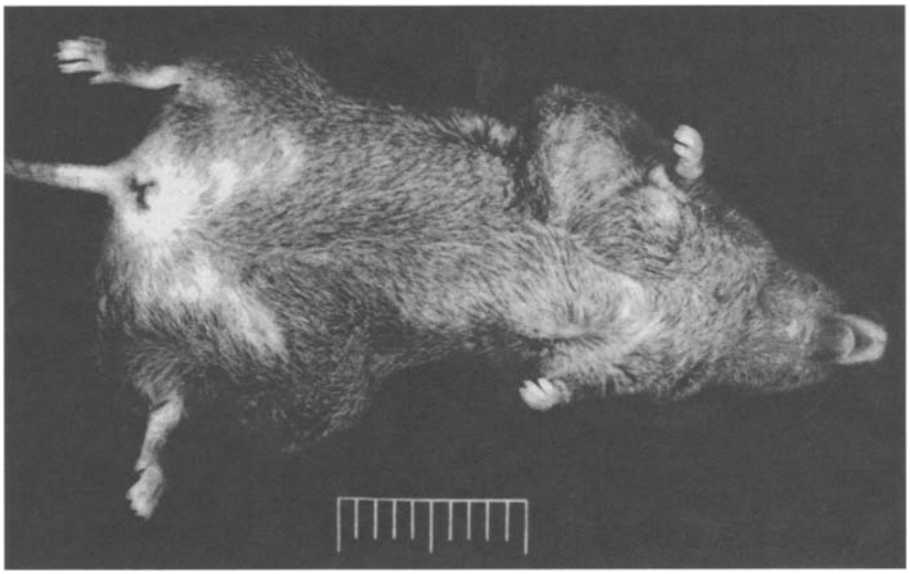

Fig. 58. Mammary tumors in a C3H mouse.

trabecular. Large carcinomas also may contain hemorrhage and necrosis. Carcinomas also may metastasize to the lungs.

4. Lung

Primary respiratory tumors of mice occur in relatively high frequency. It has been estimated that more than 95% of these tumors are pulmonary adenomas that arise either from type 2 pneumocytes or from Clara cells lining terminal bronchioles. Pulmonary adenomas usually appear as distinct whitish nodules that are easily detected by examination of the lung surface. Malignant alveologenic tumors are infrequent and consist of adenocarcinomas and squamous cell carcinomas. They invade pulmonary parenchyma and are prone to metastasize. The prevalence of spontaneous respiratory tumors is mouse strain-dependent. For example, the prevalence is high in aging A strain mice but low in aging C57BL mice. The number of tumors per lung is also higher in susceptible mice.

Pulmonary tumors often occur as well-defined gray nodules. Microscopically, adenomas of alveolar origin consist of dense ribbons of cuboidal to columnar cells with sparse stroma. Adenomas of Clara cell origin are usually associated with bronchioles. They have a tubular to papillary architecture consisting of columnar cells with basal nuclei. Pulmonary adenocarcinomas, though comparatively rare, are locally invasive. They often form papillary structures and have considerable cellular pleomorphism.

5. Neoplasms of Other Organ Systems

Neoplasms of other organ systems are described in Jones *et al.*, 1983–1993; Maronpot *et al.*, 1999; and Percy and Barthold, 1993g.

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