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CHAPTER

3

Biology and Diseases of Mice

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OUTLINE

I. INTRODUCTION

A. Origin and History

Today's laboratory mouse, *Mus musculus*, has its origins as the 'house mouse' of North America and Europe. Beginning with mice bred by mouse fanciers, laboratory stocks (outbred) derived from *M. musculus musculus* from eastern Europe and *M. m. domesticus* from western Europe were developed into inbred strains (Table 3.1). Since the mid-1980s, additional strains have been developed from Asian mice (*M.m. castaneus* from Thailand and *M.m. molossinus* from Japan) and from *M. spretus* which originated from the western Mediterranean region.

The laboratory mouse was employed in comparative anatomical studies as early as the 17th century, but accelerated interest in biology during the 19th 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

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TABLE 3.1 Standardized Abbreviations of Names for Common Inbred Strains

Abbreviation 129	Inbred 129 strains (may include subtype, e.g., 129S6 for 129S6/SvEvTac)
A	A strains
AK	AKR strains
B	C57BL strains
B6	C57BL/6 substrains
B10	C57BL/10 substrains
BR	C57BR strains
C	BALB/c strains
C3	C3H strains
CB	CBA strains
D	DBA strains
D1	DBA/1 substrains
D2	DBA/2 substrains
HR	HRS strains
L	C57L strains
R3	RIIIS strains
J	SJL strains
SW	SWR strains

Note: *The full strain/substrain designation and source should be defined and/or referenced in publications, with abbreviations used after definition thereof.*

development of the 'modern' laboratory mouse. Research use of mice has grown exponentially during the past and 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 (Silver, 1995; Lyon *et al.*, 1996; Morse, 2007a). The current ability to create highly sophisticated, genetically 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. Genetic History

Historical reviews have documented the origins of the laboratory mouse, which extend thousands of years into antiquity (Keeler, 1931; Morse, 1978; Silver, 1995). The laboratory mouse belongs within the genus *Mus*, subfamily Murinae, family Muridae, superfamily Muroidea, Order Rodentia, and within the *M. musculus* clade collectively called the 'house mouse' (Lundrigan *et al.*, 2002). Anatomic features of molar teeth and cranial bones were traditionally used by zoologists to identify over 100 different species within the genus, and to differentiate them from other murids. Because of considerable phenotypic variation within a single *Mus* species, this approach has proven to be inaccurate, and given way to contemporary genetic analysis. The native range of the genus *Mus* is Eurasia and North Africa. Members of this genus are generally classified as aboriginal, consisting of species that live independent of humans, or commensal, which includes taxa that have coevolved and geographically radiated with human civilization since the dawn of agriculture 12,000 years before present (bp). This close association with human agrarian society gave rise to the genus name, derived from Sanskrit, *Mush*: to steal. The commensal group is known as the 'house mouse' clade, consisting of several subspecies of *Mus musculus*, including *M. m. domesticus, M. m. musculus, M. m. castaneus, M. m. bactrianus*, and a lesser known lineage, *M. m. gentilulus* (Prager *et al.*, 1998). The Japanese house mouse, *M. m. molossinus*, is a natural hybrid of *M. m. musculus* and *M. m. castaneus*. The progenitor of the *M. musculus* clade arose in the northern Indian subcontinent and diverged into genetically isolated and distinct species or subspecies due to geographic barriers (mountain ranges). There is debate whether these taxa are species or subspecies, and some have referred to them as 'incipient species,' but their genetic divergence is now blurring as they colonize the world and hybridize.

The native ranges of these taxa are important for understanding the origins of various laboratory mice, whose genomes are mosaics derived from *M.m. domesticus* (~60%), *M.m. musculus* (~30%), and *M.m. castaneus* (~10%) (Wade and Daly, 2005; Wade *et al.*, 2002). It is now apparent that the *M.m. musculus* and *M.m. castaneus* contributions to the laboratory mouse genome were primarily derived from *M.m. molossinus* Japanese fancy mice (Takada *et al.*, 2013). *Mus m. domesticus* is indigenous to western Europe and southwest Asia, *M.m. musculus* to eastern Europe and northern Asia, *M.m. castaneus* to southeast Asia, and *M.m. molossinus* to Japan and the Korean peninsula. The cohabitation of humans with commensal mice gave rise to captive breeding for coat color and behavioral variants in China over 3000 years bp. By the 1700s, mouse 'fanciers' in Asia had created many varieties of fancy mice, as did European fanciers, who subsequently acquired Asian stocks, particularly Japanese fancy mice (*M.m. molossinus*), to mix with European (*M.m. domesticus*) fancy mouse varieties. This genetic mixing for fancy variants was also occurring in the United States, and these mouse lines contributed to many of the major laboratory mice used today. Meanwhile, the European colonial expansion era contributed to the worldwide dissemination of *M.m. domesticus*, which now occupies every continent of the world. It is well documented that wild-caught *M.m. domesticus* also contributed to the genetic composition of fancy and laboratory mice on multiple occasions.

Despite their diverse genetic origins and phenotypic differences, most laboratory mouse strains are closely

related, since many were derived from a genetically mixed but small number of fancy mice from a single mouse breeder (Abbie Lathrop's Granby Mouse Farm, Massachusetts) at the beginning of the 20th century. Most inbred laboratory mice share a common maternal mitochondrial genome derived from *M.m. domesticus* (Ferris *et al.*, 1982; Yu *et al.*, 2009), and a common Y chromosome contributed by *M.m. musculus* (Bishop *et al.*, 1985) through its contribution to the genome of *M.m. molossinus* (Nagamine *et al.*, 1992). Thus, the most inclusive name that can be assigned to the genetically mosaic laboratory mouse is *M. musculus*, the over-arching name for the entire commensal clade. There are exceptions, however. C57BL/6 mice contain minor genetic elements derived from *M. spretus* (Hardies *et al.*, 2000), and a number of wild aboriginal species that are not members of the *M. musculus* clade, including *M. spretus, M. caroli*, and others, have been established as inbred lines of mice.

C. Genetics

Genetic mapping in mice began in the early 1900s with a focus on inheritance of coat color. 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 genetic research (Table 3.1) (Lyon *et al.*, 1996). Mice have 20 pairs of telocentric chromosomes that are differentiated by their 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 be located physically on chromosomes by fluorescent *in situ* hybridization (FISH). 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 (Moore and Nagle, 2000). For more information on comparative genomics, see Chapter 35 *Animal Models in Biomedical Research*, subsection C.

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 critical immune responses, 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. The *H2* complex contains several loci, including K, D, L, I-A, and I-E. Inbred strains of mice, being homozygous, each have unique sets of *H2* alleles, termed H2 *haplotypes.* For example, the BALB H2 haplotype is $H2^d$ and the C57BL H2 haplotype is $H2^b$. The *International ImMunoGeneTics (IMGT) Information System* provides details on H2 haplotypes for various inbred mice (www.imgt.org/IMGTrepertoireMHC/Polymorphism/haplotypes/mouse/MHC/Mu_haplotypes.html). 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 (Stuart, 2010). Non-MHC or minor histocompatibility systems also are under active study (Roopenian *et al.*, 2000).

Mouse genomics have accelerated tremendously in the last two decades, heralded by the development of a robust physical map and high-quality genome sequence of the C57BL/6J mouse in 2002 by the *International Mouse Genome Sequencing Consortium* (Waterston *et al.*, 2001). The *Mouse Genomes Project*/Wellcome Trust Sanger Institute is extending this effort to include the genomic sequences of 17 key mouse strains. Completed and evolving sequence data are available through the *European Nucleotide Archive* (www.ebi.ac.uk/ena/home). The burgeoning numbers of inbred mouse strains, natural mutants, induced mutants, transgenic lines, and targeted mutant lines of mice are cataloged in the *Mouse Genome Informatics* (MGI) database: http://www.informatics.jax.org/mgihome). The growing number of mutant mice has fostered the development of a number of mouse repositories, from which specific mice can be located and acquired. In the United States, there are four regional National Institutes of Health (NIH)-supported *Mutant Mouse Regional Resource Centers* (http://www.mmrrc.org), which link to international repositories in Europe, Japan, China, Australia, and Canada, as well as additional resource programs in the United States through the *International Mouse Strain Resource* (IMSR; http://www.informatics.jax.org/imsr/index.jsp) for depositing, archiving, and distributing mutant mouse and embryonic stem cell lines to the scientific community. In addition to numerous mutant mice produced independently by scientists in various academic institutions, three major targeted gene knockout programs, all utilizing C57BL/6N embryonic stem cells, are under way internationally, and funded by the NIH, the European Community, and Genome Canada (Collins *et al.*, 2007; Skarnes *et al.*, 2011). These include the *Knock Out Mouse Project* (KOMP; http://www.knockoutmouse.org), the *European Conditional Mouse Mutagenesis Program* (EUCOMM; http://www.eucomm.org), and the *North American Conditional Mouse Mutagenesis Project* (NorCOMM;http://norcomm.phenogenomics.ca/index.htm). These mouse lines will be available through

three distribution centers: the *German Resource Center for Genome Research* (RZPD; http://www.rzpd.de), the KOMP repository (https://komp.org), and the Canadian Mouse Consortium (CMC; http://www.mousecanada.ca/index.htm). The repositories are all linked to the IMSR, and provide access to mice, germplasm, genomic detail, and phenotypic data. Genetic, genomic, and biological data are also available through the *International Mouse Phenotyping Consortium* (IMPC; www.mousephenotype.org) and the *Mouse Genome Database* (MGD; http://www.informatics.jax.org) (Eppig *et al.*, 2012).

D. Breeding Systems

Inbreeding is a fundamental genetic tool applied to the laboratory mouse and detailed information is available on the web (Table 3.2). The first inbred strain (DBA) was developed by C.C. Little in 1909, with the subsequent creation of over 1000 inbred strains and stocks of mice (Festing, 1996). Genetic origins, basic characteristics, references, and breeding performance of inbred strains of mice are available through Michael Festing's online version of *Inbred Strain Characteristics* (http://www.informatics.jax.org/external/festing/mouse/STRAINS.shtml). Overviews of genetic manipulation for the creation of different types of mice are available (Lyon *et al.*, 1996; Silver, 1995).

Inbred mouse lines are termed *strains*, and are achieved by 20 or more brother × sister (filial; F) generations (Table 3.3). Mice within an inbred strain, for practical purposes, are genetically identical (*syngeneic* or *isogenic*) to other mice of the same strain and sex. Because of residual heterozygosity, a strain is not *fully inbred* until after 60 F generations. Most commonly used inbred mouse strains represent 200 or more F generations, providing a high degree of experimental reproducibility. The mouse genome is not static, so when branches of an inbred strain are separated, spontaneous mutations, residual heterozygosity, and retroelement integrations result in genetic differences. Therefore, if branches of an inbred strain are separated before F_{40}, if branches have separated for 100 generations, or if genetic differences arise, the different branches become *substrains.* The same holds true if branches of a substrain diverge, resulting in substrains of the inbred substrain.

When two inbred mouse strains are crossed, the F_1 hybrids are genetically identical to one another (isogenic), but maximally heterozygous (with chromosomes of each chromosomal pair separately contributed by each parental strain), whereas F_2 hybrids are maximally genetically diverse from one another (with chromosomes of both chromosomal pairs containing a mixture of contributions from each parental strain). With each subsequent F generation, mice once again approach inbred status. This technique is used for creating *recombinant inbred* (RI) strains.

RI strains are sets of inbred strains of mice derived from crossing two inbred strains, and developed by single-pair random matings of sibling mice from the F_2 generation, thereby creating separate breeding lines. Each line created is maintained separately, and then propagated by brother–sister matings for 20 generations, with each line becoming a separate inbred strain, but belonging to a set of RI strains. RI mice are useful for mapping phenotypic or quantitative traits that differ between the progenitor strains (Bailey, 1971). RI sets are generally limited to two parental strains. An ongoing international effort has been undertaken to increase allelic diversity among RI strains by creating the *Collaborative Cross* (CC) in which a panel of RI strains are being generated mixing the genomes from eight disparately related inbred (octo-parental) mouse strains, including A/J, C57BL/6J, 129S1/SvImJ, nonobese diabetic (NOD)/ShiLtJ, NZO/HlLtJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ. These eight strains capture nearly 90% of the known genetic variation present among laboratory mice. Future applications of the CC will utilize RI intercrosses of pairs of RI CC lines (Threadgill and Churchill, 2012; Welsh *et al.*, 2012).

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 another type of RI lines. They 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.

When a mutation arises spontaneously or is induced within an inbred strain, that mutant mouse becomes *co-isogenic* with the parental inbred strain, being virtually identical except for the single mutant allele. Frequently, a mutation that arose in one inbred strain may be desired within the genetic background of another inbred strain. This can be accomplished by *backcrossing*, in which an F_1 hybrid is created by mating the donor mutant strain to the desired background strain, with subsequent matings to the background strain while retaining the mutant locus. After 10 backcross generations (*N* generations), the mutant mouse line is now *congenic* to the background inbred strain. Backcrossing to create congenic strains of mice has been used extensively when targeted mutations have been induced in 129 embryonic stem cells, with backcrossing onto C57BL/6 inbred mice. Congenic mice are never co-isogenic, as the preserved locus in a congenic mouse is invariably surrounded by flanking DNA, which may significantly influence phenotype (Linder, 2006).

TABLE 3.2 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.shtm
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
NIH Mutant Mouse Region Resource Center	https://www.mmrrc.org
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/cancerlinks.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	http://genex.hgu.mrc.ac.uk
DATABASE PROJECT	
UCD Medpath Transgenic Mouse Searcher 2.0	http://www-mp.ucdavis.edu/personaltgmousel.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://anatoM.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/modeIs/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

(*Continued*)

TABLE 3.2 (Continued)

Internet resource	Web address
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/focusIink
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

TABLE 3.3 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: Cross between two inbred strains followed by F_1 brother-sister mating for >20 generations to obtain F_2; RI strains are inbred after additional 20 generations of brother-sister matings.	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)*

In contrast to inbred mice, *outbred* mice are genetically heterogeneous and are maintained by breeding systems that intentionally minimize inbreeding. Outbred mice are called *stocks*, which are defined as a closed population (for at least four generations) of genetically variable mice that are bred to maintain maximal heterozygosity. Outbred mice may be used when high genetic heterogeneity is desired or for experiments requiring large numbers of mice. Outbreeding can be achieved only in a large breeding population using a systematic breeding scheme, or randomized selection of breeders from the population. 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 a population of 25 breeding pairs, e.g., heterozygosity will decrease at 1% per generation with standard randomization techniques. Random breeding involves the statistically random selection of breeders by using a random numbers table or computer program. An outbreeding 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 *n*th generation. Another 'circular' set of cages serves as the breeding nucleus for the $n + 1$ generation. Each mated pair in the *n*th generation contributes one female and one male to the $n + 1$ generation. Outbreeding is accomplished by assigning the female and male derived from each *n*th generation cage to different cages in the $n + 1$ generation.

Most outbred mouse stocks are of 'Swiss' origin, derived from nine mice imported to the United States in 1926, and are therefore quite homogeneous genetically (Chia *et al.*, 2005). Various lines of these mice have been maintained at different institutions, giving rise to numerous closely related stocks. Although considered outbred, they have a high degree of homozygosity, exemplified by the fact that many Swiss mouse stocks are blind due to the homozygous recessive *rd1* allele (Serfilippi *et al.*, 2004b). It is preferable to ensure genetic heterogeneity by intercrossing multiple inbred strains to achieve heterogeneity with known genetic input. In that regard, the *Diversity Outbred* mouse has been developed, which is a heterogeneous stock derived from the same eight founder inbred strains of the CC (Churchill *et al.*, 2012).

Additional types of inbred mice are utilized in research, including *consomic* and *conplastic* strains. Consomic strains, also known as chromosome substitution strains, are inbred mice that are congenic for entire chromosomes, and are useful for studying polygenic traits (Singer *et al.*, 2004). Conplastic mice are inbred mice that are congenic for different mitochondrial genomes (mtDNA) contributed by other inbred strains, other subspecies, or other species of *Mus* (Yu *et al.*, 2009).

E. Induced Mutant Mice (Genetically Engineered Mice)

In addition to spontaneously occurring mutations that are maintained as co-isogenic strains (such as the C57BL/6 beige mouse), mutant lines of mice have been created by radiation mutagenesis, chemical mutagenesis, or transgenesis. Radiation was one of the earlier methods for *in vivo* mutagenesis (Silver, 1995), but *in vitro* radiation of embryonic stem (ES) cells is also performed (Thomas *et al.*, 1998). Chemical mutagenesis involves *in vivo* treatment of male mice or *in vitro* treatment of ES cells with mutagenic chemicals such as ethylmethanesulphonate (EMS) or *N*-ethyl-*N*-nitrosourea (ENU), which induce point mutations in DNA (O'Brien and Frankel, 2003; Justice *et al.*, 1999, 2000).

Technically, a *transgenic* mouse is any mouse in which foreign DNA has been integrated into its genome, regardless of method. However, the term *transgenic* commonly refers to mice that are genetically altered by *additive transgenesis* through microinjection of foreign DNA into the pronucleus of a fertilized egg. Each ensuing embryo results in a genetically different *founder* mouse, since the transgene is integrated in random sites of the genome of each founder mouse. Since the injected DNA is not homologous to the mouse genome and is not an allele, transgenic founders are *hemizygous* (rather than *heterozygous*) for the transgene until the mice carrying the transgene are bred into homozygosity for the transgene. Transgenes typically integrate as tandem repeats, copy numbers affect phenotype of each founder, and may be lost in subsequent generations, thereby changing the phenotype of the mouse line (Tinkle and Jay, 2002). Transgenes are often constructed with an upstream *promoter*, which confers widespread (ubiquitous) or tissue-specific expression of the cDNA, so that the transgene expression pattern reflects the expression pattern of the promoter. Transcriptional regulation of the transgene can be *inducible* by drug-dependent regulatory control, such as the widely used tetracycline (*tet*) regulatory system, in which treatment of mice with tetracycline or doxycycline induces up- or down-regulation of the transgene (Jaisser, 2000).

ES cells are used for the less efficient integration of genetic material by homologous DNA recombination, but allow large-scale screening of ES cell clones for transformation. Integration can be achieved in a random fashion by *gene trapping*, or by *targeted mutation*. Both methods involve homologous DNA recombination. Gene trapping is a high-throughput approach that randomly introduces insertional mutations within the genome. Vectors contain a gene trapping cassette with a promoter-less reporter gene and/or selectable genetic marker flanked by an upstream 3′ splice site and a downstream termination sequence. When inserted into an

intron of an expressed gene, the gene trap is transcribed from the endogenous promoter of that gene. Gene traps simultaneously inactivate and report the expression of the trapped gene at the insertion site, and provide a DNA tag for the rapid identification of the disrupted gene (Skarnes *et al.*, 2011).

Targeted gene mutations are achieved by homologous recombination of specific sites within the genome of ES cells. Homologous sequences flank the upstream and downstream regions of the targeted gene, and the construct between the flanking sequences may inactivate (*knock out*) or replace (*knock in*) a gene, and typically contains a reporter gene to track the integration. A variation on this approach is site-specific recombinase (SSR) technology. Two of the most common recombinases are Cre from the coliphage P1 and FLP from *Saccharomyces cerevisiae*. Cre and FLP mediate recombination between target sites, termed *loxP* and *FRT*, respectively. For example, Cre *loxP* target sites are engineered to flank the gene target, which can be used in different ways to achieve different outcomes (*conditional mutations*), depending upon the orientation and location of the flanking *loxP* sites. If the *loxP* sites are oriented in opposite directions, Cre recombinase mediates inversion of the floxed segment. If the *loxP* sites are on different chromosomes (*trans*), Cre recombinase mediates a chromosomal translocation. If the *loxP* sites are oriented in the same direction on the same chromosome (*cis*), Cre recombinase mediates deletion of the floxed segment. Once the floxed mutation is created in ES cells, the transformed ES cells are developed into a mouse with the conditional mutation. The conditional mutant mouse is then genetically crossed with a Cre transgenic mouse, in which Cre recombinase is under the control of a ubiquitous or tissue-specific promoter. Wherever and whenever Cre is expressed, Cre recombinase will recognize and recombine the *loxP* sites. This approach can include insertion of reporter genes and selectable markers, and can be under the control of inducible gene expression systems (http://www.eucomm.org/docs/protocols/mouse_protocol_1_Sanger) (Nagy, 2000).

ES cells are pluripotent with the full genetic capacity to develop into mice when implanted into the blastocyst of a developing embryo. Interest in 'embryonal carcinomas' (teratomas) that arose in relatively high frequency in the testes of 129 mice and early gene transfer experiments in the late 1970s and early 1980s led to the development of ES cell lines derived from several different 129 strains. This early emphasis on teratomas prompted creation of 'better' 129 mouse lines that were more prone to development of testicular teratomas, resulting in genetic corruption of the 129 mouse (Simpson *et al.*, 1997; Threadgill *et al.*, 1997). This realization gave rise to the need to revise 129 mouse nomenclature (Festing *et al.*, 1999). This was necessary because genetic variation significantly impacts homologous recombination in order to match genome sequence of the ES cell line with the mouse from which it was derived. ES cells can be created from any mouse strain or hybrid, but 129 ES cell lines have been commonly used. Recent international knockout mouse program efforts use C57BL/6N ES cells.

Transformed ES cells are microinjected into the inner cell mass of recipient blastocysts, which are then implanted into the uteri of pseudopregnant surrogate mothers. The pups that are born are composed of a mixture of cells derived from recipient blastocysts and the transformed ES cells (*chimeras*). The goal is for male chimeric progeny to produce spermatozoa of ES cell origin (containing the mutation), in order to create F_1 progeny by mating the chimera with the desired background strain (http://www.eucomm.org/docs/protocols/mouse_protocol_1_Sanger). For this reason, most ES cell lines are XY, which favors 129 male chimerism. If the ES cells are of 129 (or other) strain origin, the chimeras are often bred to a desired background mouse strain (commonly C57BL/6) and backcrossed for N10 generations, thereby creating *congenic* inbred mouse lines. Recent international knockout mouse efforts utilize C57BL/6N ES cells, so that chimeric males are bred directly with C57BL/6 mice, thereby creating *co-isogenic* lines. The latter approach saves time and money, and creates a more genetically refined mutant mouse. An alternate approach is to allow ES cells to aggregate with a developing embryo to form blastocysts in culture (aggregation chimera), then implant the chimeric blastocysts (Tanaka *et al.*, 2001).

RNA interference (RNAi), which functions through short double-stranded RNA (dsRNA), has also been utilized to produce transgenic mice, known as gene *knockdown* mice (Gao and Zhang, 2007; Peng *et al.*, 2006). The dsRNA is enzymatically processed into small molecules, termed small interfering RNA (siRNA), which find homologous target mRNAs, resulting in interference. This phenomenon is believed to be a self-defense mechanism against viral infection. In order to adapt this approach to generation of transgenic mice, small hairpin RNA (shRNA) can be expressed in the same way as other transgenes in mice, resulting in processing of the shRNA into siRNA with gene-silencing effects. Constructs are introduced into mouse ES cells by electroporation or lentiviral infection. This method can be embellished conditionally, as with other transgenes. Although RNAi knockdown mice are genetically stable, RNAi-mediated transgenesis is never complete, has variable tissue expression, and cannot induce point mutations (Peng *et al.*, 2006).

1. *Engineered Endonuclease Technologies*

Recent advances in engineered endonuclease (EE) technology, including zinc finger nucleases (ZFNs),

transcription activator-like effector nucleases (TALENs), and RNA-guided endonucleases (RGENs), have revolutionized the field of transgenics (Sung *et al.*, 2014; Wijshake *et al.*, 2014; Gaj *et al.*, 2013). ZFNs and TALENs consist of engineered proteins that target DNA fused to the nonspecific endonuclease, Fok1 (Cathomen and Joung, 2008; Joung and Sander, 2013). ZFNs are comprised of three to six tandem zinc finger proteins, each of which targets a specific 3 bp nucleotide sequence. Paired ZFNs are generated, with each half of the pair targeting opposite DNA strands, allowing dimerization of Fok1 which is required for introduction of double-stranded breaks (DSBs) in the DNA of interest (Cathomen and Joung, 2008). TALENs function similarly, but are composed of tandem repeats of 33–35 amino acids, each with nucleotide specificity occurring in two hypervariable amino acids, the 'repeat variable di-residue (RVD)', at positions 12 and 13 (Joung and Sander, 2013).

In contrast to ZFNs and TALENs, clustered regularly interspaced short palindromic repeats (CRISPRs) paired with CRISPR-associated (CRISPR/Cas) systems are RGEN systems that target specific DNA sequences. Cas proteins, rather than Fok1, produce DSB (Hsu *et al.*, 2014).

DSB generated by EE are repaired by host cells by either nonhomologous end joining (NHEJ) or, less commonly, by homologous recombination (HR). NHEJ is an error-prone repair system and results in insertions or deletions (indels) with a relatively high frequency, which can result in gene disruption. HR is a less common repair pathway, but certain manipulations of the engineered nucleases can increase HR efficiency. For example, nucleases can be engineered to generate a break in a single strand of DNA rather than inducing DSB, and the resulting nickases increase the incidence of HR with high fidelity (Gaj *et al.*, 2013; Wijshake *et al.*, 2014). HR allows for the introduction of donor DNA to generate knock-ins, specific point mutations, or for the generation of larger modifications such as insertions of loxP sites (Brown *et al.*, 2013; Wijshake *et al.*, 2014).

Vectors encoding the EE can be injected into mouse embryos by pronuclear injection of DNA, intracytoplasmic injection of RNA, or transfection of mouse ES cells (Sung *et al.*, 2014; Wijshake *et al.*, 2014). One advantage of EE technologies over more traditional transgenic methods is the ability to target DNA and induce mutations in any background strain of mouse negating the need to backcross onto the desired strain. Multiple genes can be targeted with CRISPRs simultaneously, thus avoiding the need to cross single knockout animals (Zhou *et al.*, 2014). In addition, it is possible to obtain bi-allelic mutations in some cases, allowing for the generation of functional gene knockout animals in a single generation (Zhou *et al.*, 2014; Wijshake *et al.*, 2014). Vectors for generating EE are available through plasmid repositories; websites are available to assist in identifying appropriate DNA sequences to target; and multiple websites post protocols for generating the various types of engineered endonucleases (Xie *et al.*, 2014; Sander *et al.*, 2010; Bae *et al.*, 2014; Reyon *et al.*, 2011; Herscovitch *et al.*, 2012; Wolfson, 2013). CRISPRs tend to be particularly cost effective and easy to design, with minimal restrictions for targeting specific DNA sequences.

F. Nomenclature

There are currently more than 1000 separate outbred stocks and traditional inbred strains, often with multiple substrains (Table 3.4). In addition, there are thousands of induced mutant strains. Therefore, it is critical that strain or stock designations be complete and accurate to avoid semantic and genetic confusion, and to ensure reproducibility of research results. As an example of substrain variation that makes precise nomenclature important, CBA/J mice are homozygous for the retinal degeneration allele (*rd1*), whereas CBA/CaJ mice do not carry this allele. The *International Committee on Standardized Genetic Nomenclature for Mice and Rats*, established in the early 1950s, is responsible for genetic nomenclature rules. The rules are available online at the MGI website (http://www.informatics.jax.org/mgihome/nomen).

Inbred mouse strains are designated by a series of capital letters and/or numbers, which often provide a shorthand description of the origin and history of the strain. The C57BL/6J mouse serves as an example. The inbred strain C57BL originated from Abbie Lathrop's female 57 (and male 52) at the Cold Spring Harbor Laboratory (C), and was the black (BL) line from this female. Early in their history, inbred C57BL mice split into major substrains, e.g., C57BL/6 and C57BL/10. Substrains are identified by appending a forward slash (/) after the inbred strain name. Since 1950, uniform international nomenclature has been built upon these historical names, so that substrains of an inbred strain are now designated using lab codes that are registered in the *International Laboratory Code Registry* maintained at the *Institute for Laboratory Animal Research* (ILAR) of the National Academies (dels.nas.edu/global/ilar/Lab-codes). Laboratory codes are composed of one to five letters that identify an institute, laboratory, or investigator. Each lab code starts with an uppercase letter, followed by lowercase letters if more than one letter is used (such as N, J, Jci, Crl, and Tac). The J in C57BL/6J means it is a substrain maintained at the Jackson Laboratory (J). Another common substrain of C57BL/6 mice is C57BL/6N, which is maintained at NIH (N). Substrains can be cumulative, reflecting the genetic history of the mouse strain. For example, there are a number of C57BL/6J substrains (such as C57BL/6JJci and C57BL/6JJmsSlc), and a number of C57BL/6N substrains (such as C57BL/6NJci, C57BL/6NCrlCrlj,

TABLE 3.4 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*/+	Co-isogenic segregating inbred mutant strain carrying the ashen (*ash*) mutation, which arose on C3H/HeSn
C57BL/6J-$^{Tyrc\text{-}2J}$/+	Co-isogenic 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-D4*Mit25-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 one or two 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 'knock-in' 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

and C57BL/6NTac). Significant differences may exist among these substrains (Mekada *et al.*, 2009). Thus, a string of substrain designations indicate the genetic progression of the substrain, which can be identified when reading the entire strain name. This nomenclature is highly nuanced, as C57BL/6NCrlCrlj mice, whose last letter is a lowercase j, are not a substrain maintained at the Jackson Laboratory (J), but rather at Charles River Japan (Crlj), underscoring the importance of upper- and lowercase lettering in rodent nomenclature. BALB/c mice are another popular inbred strain with numerous substrains. Like the '6' in C57BL/6, the 'c' that follows

the '/' in BALB/c is a lowercase letter because of historical precedent. Subsequent substrains follow accepted nomenclature, e.g., BALB/cByJ and BALB/cAnN.

Hybrids of two inbred strains are often used in research, and are particularly common with engineered mutations that are created in 129-derived ES cells, followed by intercrossing the 129 chimeric mice with C57BL/6 or other background strains of mouse. When an F_1 hybrid is created, the female partner is listed first, e.g., a C57BL/6J × 129S2/SvPas hybrid would be designated: C57BL/6J129S2/SvPasF1. RI strain sets that are derived from two parental inbred strains are identified by an X between the two parental strains followed by a hyphen designating the specific RI line, e.g., C57BL/6JXDBA/2J-1, C57BL/6JXDBA/2J-2, etc. CC RI strains do not use the X between the parental strains because they are derived from eight parental strains, so they are designated CC-1, CC-2, etc. In order to simplify the complexity of this nomenclature, abbreviations are used for common inbred strains and substrains of mice (Table 3.4), but it is important to include the full genetic nomenclature in publications. Using the abbreviated nomenclature, C57BL/6J129S2/SvPasF1 mice would be B6129F1 and C57BL/6JXDBA/2J-1 RI mice would be BXD-1. Parental order is an important consideration in nomenclature, as a B6129 mouse is genetically different from a 129B6 mouse due to mitochondrial DNA (from the female) and Y chromosome (from the male) differences.

Mutant genes are designated by a brief abbreviation for the mutation (e.g., *bg* for beige which arose at the Jackson Laboratory, *J*). The symbol for the parent gene is noted in italics, starting with an uppercase letter (e.g., *Lyst*) and the mutant allele is designated in superscript (e.g., $Lyst^{bgJ}$). Thus, the beige mutation arose in C57BL/6J mice, so that C57BL/6J beige mice, which are co-isogenic with C57BL/6J mice, are designated C57BL/6J-$Lyst^{bgJ}$.

A *transgenic strain* is designated by the strain and substrain name, followed by a symbol for the transgene. Transgene symbols take the form Tg(YYY)#Zzz, where 'Tg' indicates transgenic, YYY defines the transgene as 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. For example, FVB/N-Tg(MMTV-Erb2)1Led mice are inbred FVB/N mice in which the rat *Erb2* gene was introduced under control of the mouse mammary tumor virus (MMTV) LTR promoter (MMTV-Erb2), the first line (1) created in the laboratory of Phil Leder (lab code Led). When a transgene causes an insertional mutation in an identified endogenous gene, the mutant allele of the gene is designated by using the gene symbol and an abbreviation for the transgene as a superscript (-Abc^{tg1Zzz}). A targeted mutation, or knockout, is designated by the mutated gene with the identification of the mutational event as a superscript. For example, $Cftr^{tm1Unc}$ is a targeted mutation (tm), first line (1) created at the University of North Carolina (lab code Unc) in the cystic fibrosis transmembrane regulator gene (*Cftr*). If the mutant allele was created by gene trap, the superscript would read 'Gt' in lieu of Tg. A gene replacement, or knock-in, uses similar nomenclature; $Myf5^{Myod}$ indicates that the *Myf5* gene was replaced by the *Myod* gene.

Congenic mice are often derived from 129 ES cells, backcrossed onto a background strain, such as C57BL/6. Under such circumstances, when the backcross generation is at N10, the '.' symbol is used between the background inbred strain and the donor strain (e.g., C57BL/6N.129P2/OlaHsd-Abc^{tm1Zzz}, abbreviated as B6.129- Abc^{tm1Zzz}. When backcrossing is incomplete but at the N5 generation, the mouse is an *incipient congenic*, designated with a ';' in lieu of a '.': B6;129- Abc^{tm1Zzz}. If the background strain is mixed genetic origin, it is designated STOCK.129- Abc^{tm1Zzz}. If the donor strain is mixed origin, it is designated 'Cg'. For example, B6.Cg-Abc^{tm1Zzz} outbred 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 (Swiss) outbred stock maintained by Harlan Sprague Dawley (Hsd).

The above overview covers the nomenclature of commonly encountered types of mice. There are numerous additional specifications for nomenclature of mice. Details are available at the MGI website (http://www.informatics.jax.org/mgihome/nomen).

G. Housing, Husbandry, and Nutrition

1. *Housing and Husbandry*

Optimum housing conditions and husbandry practices for research mice should be guided by program requirements to ensure biosecurity, occupational health, efficient use of equipment, labor and financial resources, behavioral needs of mice, and investigator needs for consistent colony maintenance, including standardized husbandry practices and nutrition. The emerging interest in the mouse microbiome in combination with the immune competency of diverse genetically engineered mouse strains demands high standards of mouse care. Mouse colonies are optimally maintained as specific-pathogen-free (SPF) which obligates veterinary and facility management to exclude specific organisms. Housing options for SPF immunocompetent mice typically include static or individually ventilated microisolator cages, which differ significantly in cost and labor required to maintain. Severely immunodeficient strains such as NOD.Cg-*Prkdcscid Il2rgtm1Wjl*/SzJ (NSG) mice require staff training, caging systems and husbandry practices that minimize risk for opportunistic infections

(Foreman *et al.*, 2011). Barrier practices and microisolator techniques may include autoclaved or irradiated feed and bedding, autoclaved or acidified water, cage-to-cage transfer of mice using disinfected forceps, positive displacement change hoods, and verified sanitation of caging and equipment through tunnel or rack washers to prevent fomite transmission of infectious agents (Compton *et al.*, 2012). In addition to husbandry staff, it is critical to maintenance of colony health status that investigators who handle cages are also trained in these techniques.

The microenvironment for mice is the cage which will vary in design, size, and composition. Vendors often successfully house production colonies in open-top cages to expedite detection of pathogen transmission should a break occur. End-users usually prefer filter-top microisolator cages which prevent (at least) gross contamination between cages by fecal contamination and aerosolized debris. The objective is to keep mice in an uncrowded, socially compatible, low-odor, dry and clean environment. Ambient temperature should minimize any confounding impact on the animal model and energy expenditure for the mice, while also being suitable for staff and investigators. Shoebox static cages made of polycarbonate, polypropylene, or polystyrene plastic (in order of decreasing cost and durability) with filtered microisolator tops continue to be used for housing and breeding mice. Older cage designs are being rapidly supplanted by individually ventilated caging systems that promote the advantages of increasing housing capacity, decreasing labor costs, and mitigating exposure of mice to noxious gases such as ammonia and exposure of humans to allergens. As more advanced caging systems are developed, the level of biosecurity may be increased but at the cost of increased health surveillance efforts to detect the source of an infectious outbreak (Shek, 2008). Disposable, recyclable polyethylene caging is a recent innovation, particularly for facilities not equipped with a cage wash facility.

Animal care programs should carefully consider the necessity for housing mice on wire-mesh flooring because of injury risk to limbs and thermoregulation issues in neonates and hairless mice which are more difficult to maintain without nesting material. Solid-bottom cages should contain sanitary bedding, such as hardwood chips, paper products, or ground corn cob. Criteria for selecting bedding vary with experimental and husbandry needs. It may be preferable to irradiate or autoclave bedding, but if this is not done, the bedding should be used only after its origin and microbial content have been evaluated (Table 3.5). Germfree and gnotobiotic mice require positive pressure isolators, most usually flexible film, with additional protection provided by sterile air through high-efficiency particulate air (HEPA) filters. This equipment can be negatively pressurized when the objective is to contain known or unknown pathogens. Animal care programs should establish enrichment policies which for mice should include social housing when mice are compatible and experiments do not require single housing. Species-specific behaviors are encouraged by nesting material and hiding places such as tubes or shacks.

TABLE 3.5 Tests of Bedding Quality[a]

CHEMICAL PROPERTIES
Pesticides and polychlorinated compounds
Mycotoxins
Nitrosamines
Detergent residues
Ether-extractable substances
Heavy metals
PHYSICAL PROPERTIES
Particle uniformity
Absorptivity
Ammonia evolution
Visible trauma and irritant potential
MICROBIOLOGICAL PROPERTIES
Standard plate count
Yeasts and molds
Coliforms and *Salmonella*
Pseudomonas

[a]*Modified from Kraft (1980).*

2. Nutrition

Nutrient requirements for the mouse are influenced by genetic background, disease status, growth rate, pregnancy, lactation, and environmental factors such as ambient temperature. The best current estimate of nutritional requirements is shown in Table 3.6. Nutritional requirements for laboratory mice are also published periodically by the National Research Council and have been reviewed by Knapka and coworkers (Knapka *et al.*, 1974; Knapka, 1983). Feed intake and weight gain data are used to estimate the nutritional needs of a particular stock or strain. Mice consume about 3–5 g 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. 3.1 and 3.2).

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

TABLE 3.6 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

(*Continued*)

TABLE 3.6 (Continued)

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]*Nutrition (1977).*
[g]*Hurley and Bell (1974).*
[h]*Pleasants* et al. *(1973).*

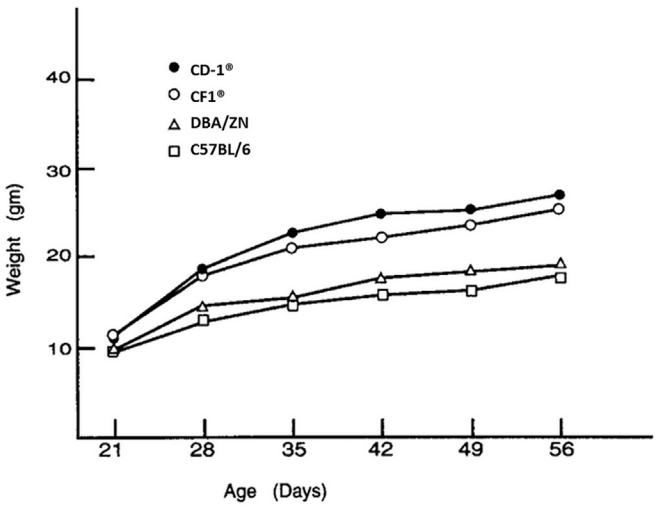

FIGURE 3.1 Growth comparison: female outbred (CD1 and CF1) and inbred DBA/ZN and C57BL/6 mice. *Courtesy of Charles River Laboratories.*

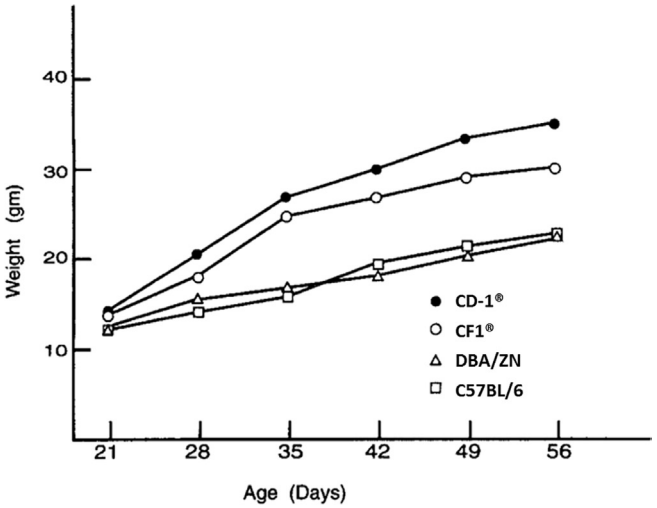

FIGURE 3.2 Growth comparison: male outbred (CD1 and CF1) and inbred DBA/ZN and C57BL/6 mice. *Courtesy of Charles River Laboratories.*

nutrients, and contaminants must be kept to a minimum (see also Chapter 29). Natural-product commercial diets for mice are usually satisfactory for breeding and maintenance. Animal care programs should avoid using fresh produce, grains, fish meal, or other supplements to minimize exposure of colonies to pathogens or harmful chemicals such as pesticide residues or phytoestrogens (Guerrero-Bosagna *et al.*, 2008).

Mouse diets can be purchased as open-formula, fixed-formula, constant nutrition, and closed-formula which

are designed to reduce variation in experimental data attributable to diet (reviewed in Barnard *et al.* (2009)). Diets are supplied in standard, irradiated, or autoclavable formulations. Irradiated diets will be virtually free of live microorganisms but have the risk of residual, radio-resistant bacteria. Autoclavable diets are higher in heat-labile nutrient content. Many programs use sterilized mouse chow exclusively to minimize risk of opportunistic infections. Because commercial diets vary in nutrient content, diets should be selected for optimal maintenance of adult mice or for growth and reproduction in breeding colonies.

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 immediately post weaning and weanlings on automatic watering systems need extra attention. Water intake will decrease in sick mice. Therefore, dosing mice with medicated water requires careful assessment of hydration and clinical or experimental efficacy of the compound administered.

II. BIOLOGY

A. Physiology and Anatomy

The main reference used to update this section of the 3rd Edition is *Volume III; Normative Biology, Husbandry and Models* in *The Mouse in Biomedical Research*, 2nd Edition, ACLAM Series published by Academic Press. Normative data on the mouse are presented in Table 3.7, and clinical chemistry reference ranges are summarized in Table 3.8.

TABLE 3.7 Normative Data for the Mouse

ADULT WEIGHT	
Male	20–40 g
Female	18–35 g
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 g/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 mmHg
Diastolic	102–110 mmHg
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 µl/g
PLASMA	
pH	7.2–7.4
CO_2	21.9 mEq/L
CO_2 pressure	40 ± 5.4 mmHg
LEUKOCYTE COUNT	
Total	8.4 (5.1–11.6) × 10^3/µl
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/µl
Packed cell volume	44 (42–44)%
Red blood cells	8.7–10.5 × 10^8/mm^3
Hemoglobin	13.4 (12.2–16.2) g/dl
Maximum volume of single bleeding	5 ml/kg
Clotting time	2–10 min
PTT	55–110 s
Prothrombin time	7–19 s

TABLE 3.8 Clinical Chemistry Reference Ranges for Adult Mice[a]

		CD-1		C57BL/6		BALB/cBy	
Analyte	**Units**	**M**	**F**	**M**	**F**	**M**	**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	–	–

(*Continued*)

TABLE 3.8 (Continued)

		Male	Female	Female
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/ml	5	20–30 fold lower	–
Albumin	mg/ml	11.9 ± 0.2	–	–

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

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, e.g., by nonshivering thermogenesis. A resting mouse acclimated to cold can generate heat equivalent to 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 h to maintain body temperature for each 1°C drop in T_A below the thermoneutral zone. 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 T_A.

Because of the 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°C 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 measured thus far. Thermoneutrality should not be equated with comfort or physiological economy. Recent data have suggested that mice housed under routine vivarium conditions are chronically cold-stressed. Mice maintained at 21°C were shown to expend more energy compared with mice housed at intermediate (26°C) and a higher temperature (31°C) with an increase in glucose utilization and activation of brown adipose tissue (David *et al.*, 2013). In contrast, other studies report 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.

2. Respiratory System

The respiratory tract has three main portions: the anterior respiratory tract consists of nostrils, nasal cavities, and nasopharnyx; the intermediate section consists of larynx, trachea, and bronchi, all of which have cartilaginous 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 (Cook, 1983) (Fig. 3.3).

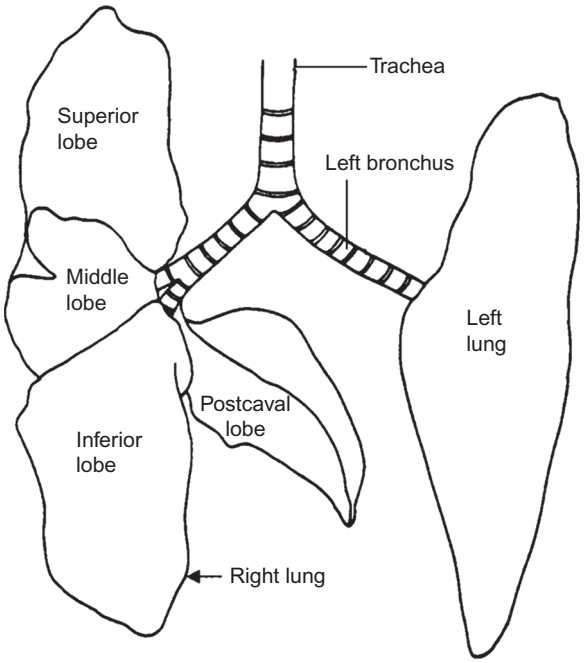

FIGURE 3.3 The five lobes of the lung. *From Cook (1983).*

A mouse at rest uses about 3.5 ml O_2/g/h, which is about 22 times more O_2/g/h 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. 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 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 3.8). 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/l, whereas humans can concentrate to a maximum of 1160 mOsm/l.

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 (GI) 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 (at least) 1000 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/g 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.

Gnotobiotic animals colonized with known microbiota have been used to great advantage as models for biomedical research (see Chapter 39). 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. Studies have quantified the regional colonization of the ASF strains along the gastrointestinal tract (Sarma-Rupavtarm *et al.*, 2004) (Fig. 3.4). Individual strain abundance was dependent on oxygen sensitivity, with microaerotolerant *Lactobacillus murinus* ASF361 present at 10^5–10^7 cells/g of tissue in the upper gastrointestinal tract and obligate anaerobic ASF strains being predominant in the cecal and colonic flora at 10^8–10^{10} cells/g of tissue.

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. With the advent of polymerase chain reaction (PCR) technology, 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 and genome sequencing indicated that each of the strains differed from its presumptive identity (Wannemuehler *et al.*, 2014). The 16S rRNA sequence of strain ASF 361 is essentially identical to the 16S rRNA sequences of the type strains of *L. murinus* and *L. animalis* (both isolated

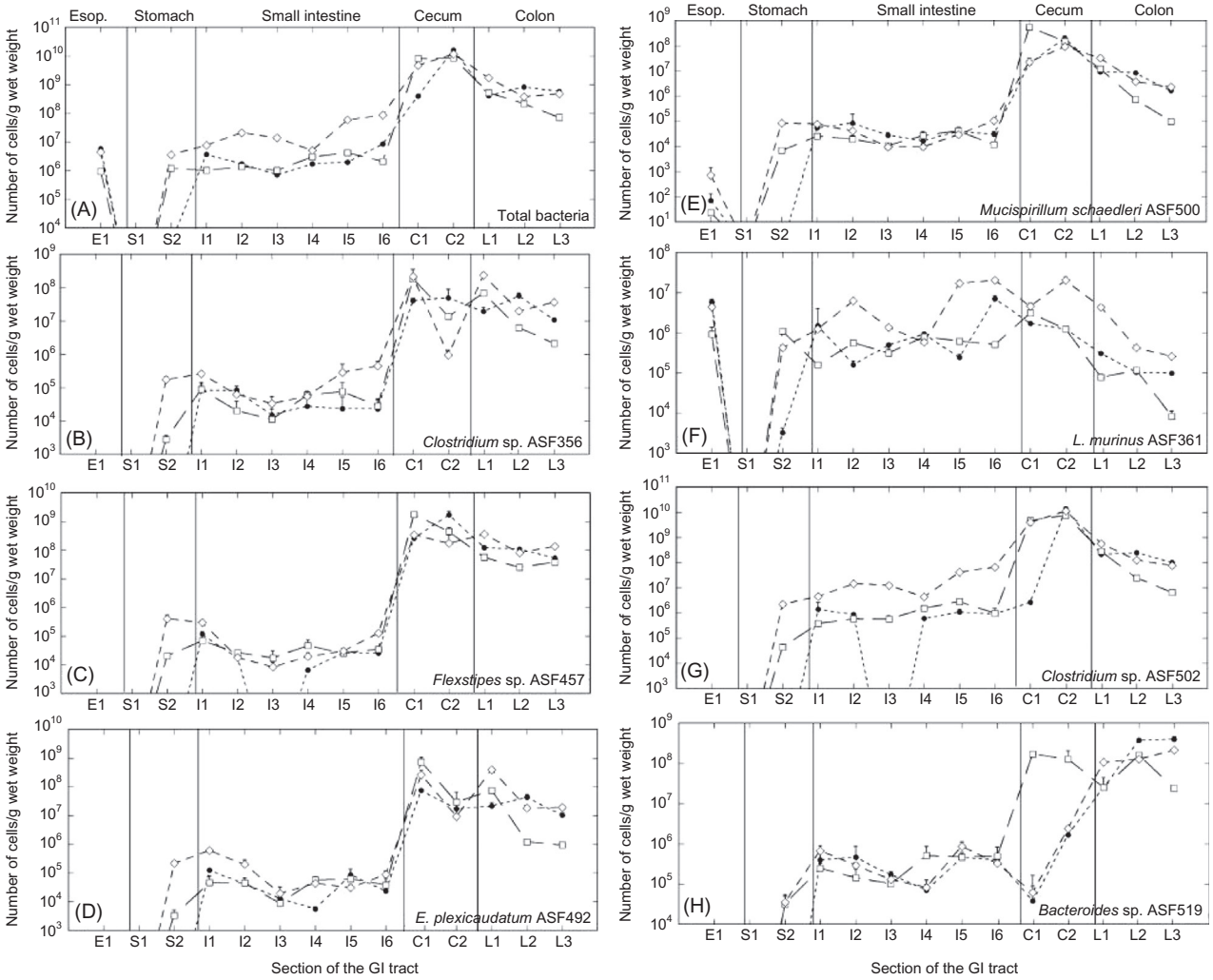

FIGURE 3.4 Distribution of ASF strains in different sections of the gastrointestinal (GI) tracts of three defined flora C.B-17 mice. The number of total bacterial cells of ASF strains (A), ASF356 (B), ASF457 (C), ASF492 (D), ASF500 (E), ASF361 (F), ASF502 (G), and ASF519 (H) in mouse 1 (solid circles), mouse 2 (open squares), and mouse 3 (open diamonds) is shown. The sections are taken from the esophagus (Esop.) (section E1), stomach (sections S1 and S2), small intestine (sections I1 to I6), ileocecal junction and apical cecum (sections C1 and C2, respectively), and colon (sections L1 to L3). *From Sarma-Rupavtarm et al.* (2004).

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 is a *Parabacteroides* sp. The spiral-shaped strain, strain ASF 457, is in the *Flexistipes* phylum, exhibits sequence identity with rodent isolates of Robertson, and has been formally named, *Mucispirillum schaedleri* (Robertson *et al.*, 2005). 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) (ASF 492 – *Eubacterium plexicaudatium*; ASF 500 – *Firmicutes* bacterium; ASF 502 and ASF 356 – *Clostridium* sp.) (Fig. 3.5). The 16S rRNA sequence information was determined by Dewhirst *et al.* (1999) and draft genome sequences for each member of ASF were recently published (Wannemuehler *et al.*, 2014). This genetic data will 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. 3.6), 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

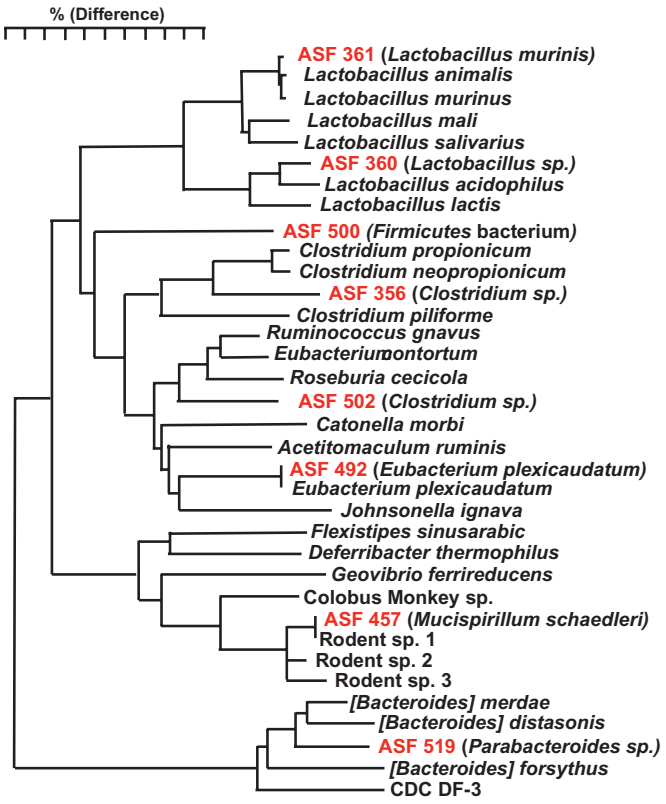

FIGURE 3.5 Phylogenetic relationships of ASF strains. From Dewhirst *et al.* (1999). Draft genome sequences of the ASF (Wannemuehler *et al.*, 2014) have identified ASF356 as *Clostridum* sp., ASF360 as *Lactobacillus* sp., ASF361 as *Lactobacillus murinus*, ASF457 as *Mucispirillum schaedleri*, ASF492 as *Eubacterium plexicaudatum*, ASF500 as *Firmicutes* bacterium, ASF502 as *Clostridum* sp., and ASF519 as *Parabacteroides* sp.

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, e.g., 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 respiratory and 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 3.7.

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.

7. Cardiovascular System

The cardiovascular system of mice is reviewed extensively by Hoyt *et al.* in the 2nd Edition of *Volume III; Normative Biology, Husbandry and Models* in the ACLAM Series *The Mouse in Biomedical Research* (Hoyt, 2007). The heart consists of four chambers, the thin-walled atria and the thick-walled ventricles (Fig. 3.7). Mice conditioned to a recording apparatus have mean systolic blood pressures ranging from 84 to 105 mmHg. 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.

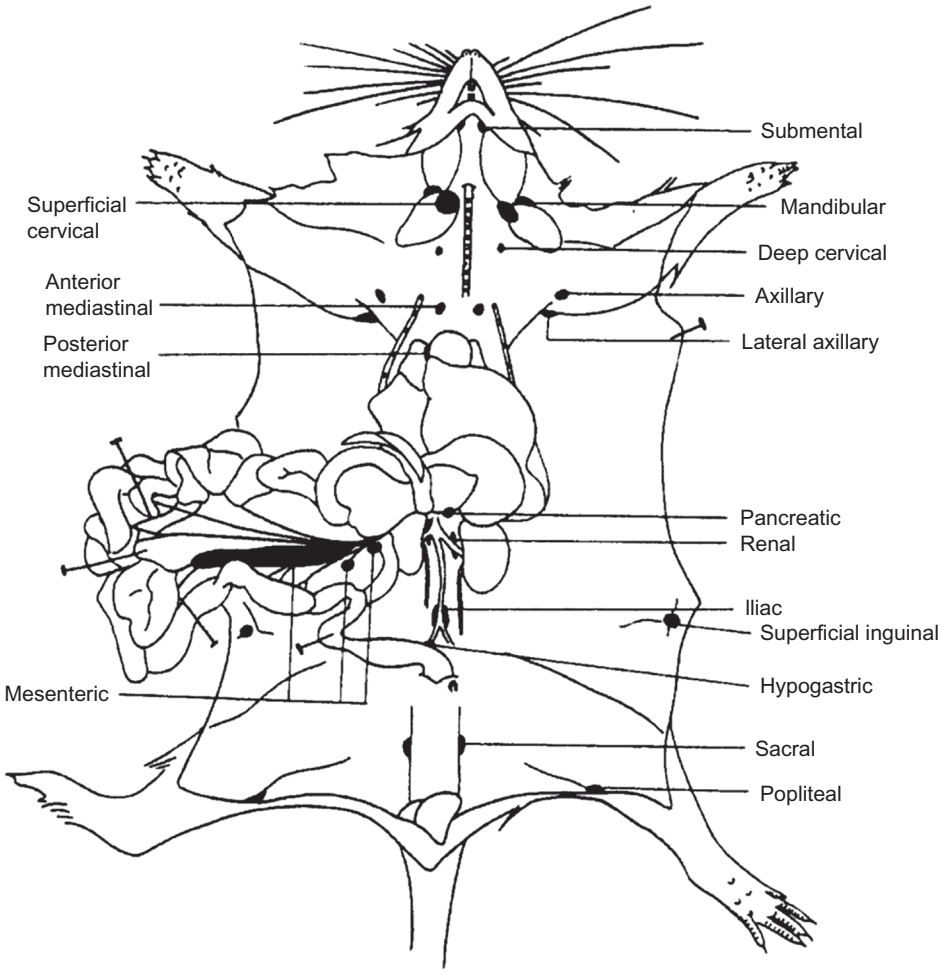

FIGURE 3.6 Lymph nodes. *Modified from Cook (1983).*

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 as documented by a detailed study of the neuroanatomy of the C57BL/6J mouse (Sidman *et al.*, 1971). More recently, gene expression patterns have been used to study the functional anatomy of the mouse brain (Bohland *et al.*, 2010). Use of wild-type and genetically modified mice in behavior, learning, and memory paradigms has exponentially increased over the last decade.

10. Genital System

The male reproductive organs consist of paired testes, urethra, penis, prostate and associated ducts and glands (Fig. 3.8). The female reproductive organs consist of paired ovaries and oviducts, uterus, cervix, vagina, clitoris, and paired clitoral glands (Fig. 3.9). 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 (Fig. 3.10). The mammary glands are often not appreciated for how far they extend over the cervical, axillary, and inguinoabdominal flank regions which become

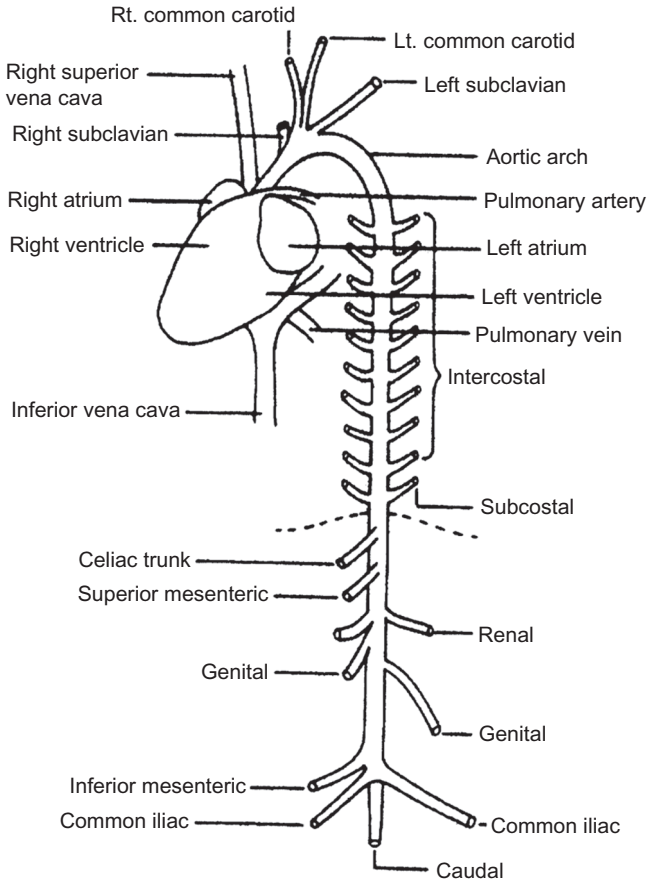

FIGURE 3.7 Heart and major vessels. *Modified from Cook (1983).*

evident when mammary neoplasia develops. Detailed techniques for manipulating gametes and embryos have been developed (Gama Sosa *et al.*, 2010).

B. Reproduction

The following section summarizes normal reproduction in the mouse. The reader is referred to a more comprehensive text in the ACLAM Series (Pritchett and Taft, 2007) and online resources such as The Jackson Laboratories publication of *The Biology of the Laboratory Mouse* (http://jaxmice.jax.org/jaxnotes/509/509j.html). External influences, such as noise, vibration, diet, light cycle, and cage density, and intrinsic factors, such as health status, genetics, and parity impact reproductive success by directly or indirectly influencing the hypothalamic–pituitary axis for hormonal control of ovarian and testicular function. Genotype also dramatically affects the reproductive performance of the mouse. Coincident with the explosion in the number of mouse strains, each with unique induced or spontaneous mutations, a sound breeding program must include training of care staff to recognize anticipated and unanticipated breeding performance and strain or stock characteristics. In the new age of genomics, older methods of confirming genetic purity of mouse lines are being replaced with formal genetic monitoring by comparing strain-specific panels of single-nucleotide polymorphisms (SNPs).

1. Sexual Maturation

Follicle-stimulating hormone promotes gametogenesis in both sexes. Luteinizing hormone promotes 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.

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 3.9). Patency of the vaginal orifice and swelling of the vulva are useful signs of proestrus and estrus (Fig. 3.11). 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 h 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

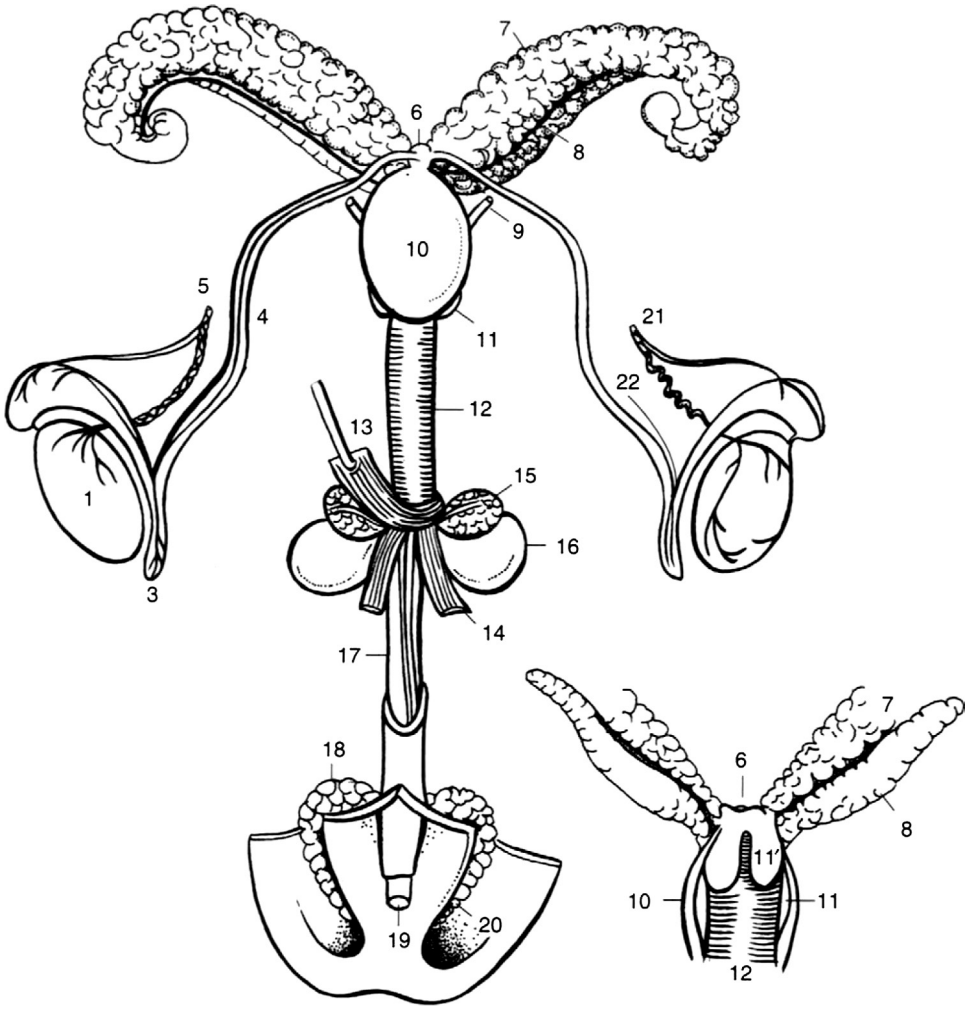

FIGURE 3.8 Reproductive anatomy of male mice. (1) testis, (2) head of epididymitis, (3) caudal epididymitis, (4) vas deferens, (5) testicular vein, (6) ampullary gland, (7) seminal vesicle, (8) anterior prostate, (9) ureter, (10) bladder, (11) ventral prostate, (11') dorsal prostate, (12) urethra, (13) bulbourethral muscle, (14) ischiocavernosus, (15) bulbourethral gland, (16) diverticulum of bulbourethral gland, (17) penis, (18) preputial gland, (19) glans penis, (20) prepuce, (21) testicular artery, and (22) vas deferens artery. *Adapted from (Komarek, 2007).*

of the light–dark cycle reverses the time of estrus, ovulation, and mating.

Pheromones (Table 3.10) and social environment also affect the estrous cycle. For example, estrus may be suppressed in group-housed female mice and reentry into estrus can be synchronized by exposure to pheromones in male mouse urine ('Whitten effect'). Once exposed to male urine, most female mice will be in estrus within 3 days with a second estrus in about 11 days. Hence, estrus can be synchronized by group-housing females prior to pairing with males. In contrast, pheromones from a strange male mouse, particularly of a different strain, may prevent implantation or pseudopregnancy in recently bred females and is known as the 'Bruce effect'. See Section II.C on Behavior for more detail on the effect of pheromones on mouse reproductive behavior.

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. 3.11). 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

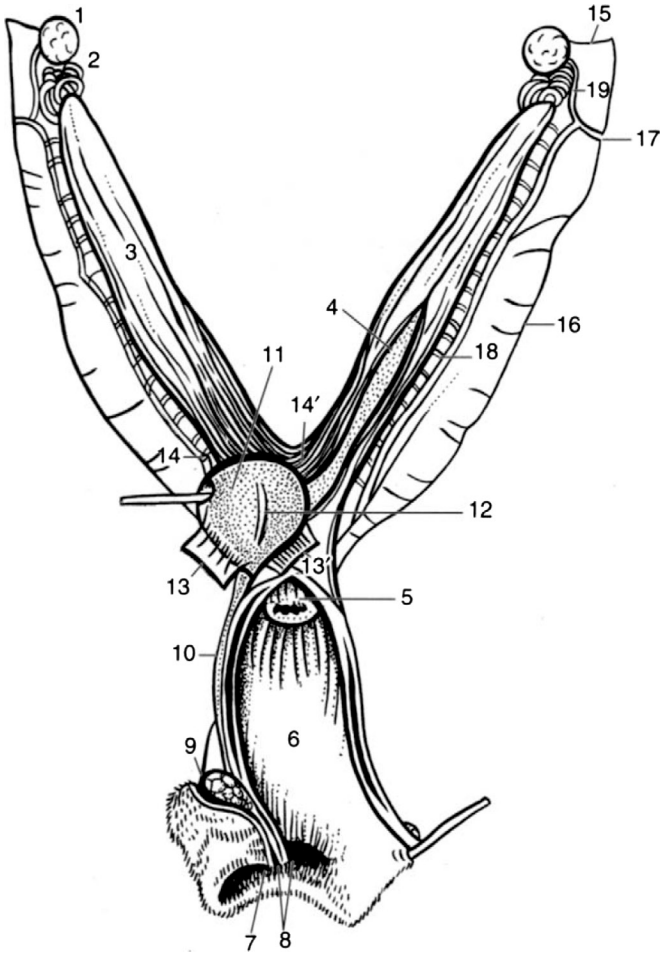

FIGURE 3.9 Reproductive anatomy of female mice. (1) ovary, (2) fallopian tube, (3) uterine horn, (4) endometrium, (5) cervix, (6) vagina, (7) vaginal vestibulum, (8) clitoris, (9) clitoral gland, (10) urethra, (11) bladder, (12) medial ligament of bladder, (13) lateral ligament of bladder, (14) left ureter, (14′) right ureter, (15) mesovarium, (16) mesometrium, (17) ovarian artery, (18) uterine horn artery, and (19) ovarian artery and vein. *Adapted from (Komarek, 2007).*

in the ampulla or the upper portion of the oviduct. Ova can be fertilized to produce normal embryos for 10–12 h 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

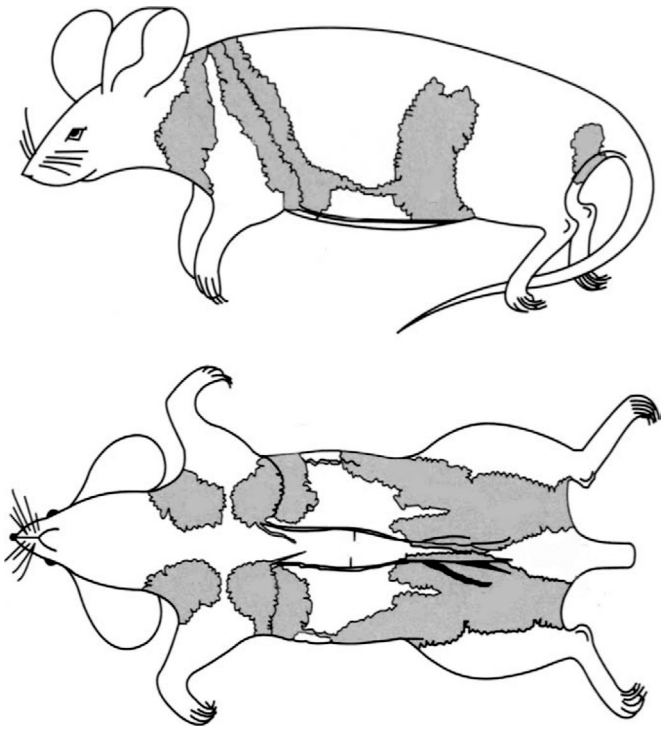

FIGURE 3.10 Distribution of mammary tissue in female mice *Adapted from Komarek (2007).*

TABLE 3.9 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 increase. 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 the 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 the 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.*

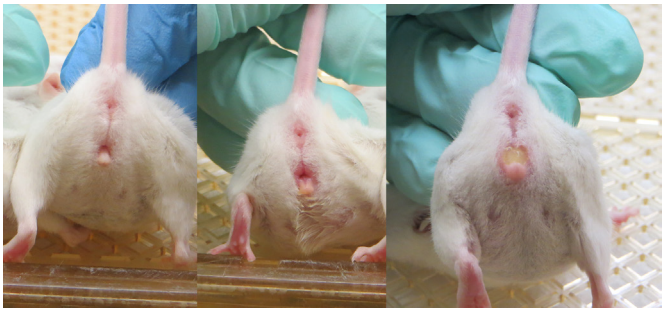

FIGURE 3.11 The female mouse on the left would likely be unreceptive to a male. The mouse in the middle is in proestrus or estrus as evidenced by erythema and edema of the vulva. The female on the right has a vaginal plug as evidence of mating the prior night. *Courtesy of the Division of Comparative Medicine.*

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 6 months of age. Average litter size is strain dependent and commonly ranges from 1 to 12 pups.

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 socially gregarious animals with strong family bonds who communicate through complex olfactory, auditory, tactile, and visual signals. Wild mice aggregate into groups called *demes* with low exchange of individuals between different groups. Each deme consists of kin-related members with a high degree of natural inbreeding,

TABLE 3.10 Select Pheromone Effects in Mice

Initiator	Effect
Male-specific major urinary protein MUP20 (darcin)	Rewarding and attractive to females[a]; intermale aggression[a]; area avoidance[b]
Male lacrimal protein ESP1	Lordosis in female mice[a]
Exocrine-gland (lacrimal) secreting peptide 22 (ESP22) produced by juvenile male mice	Inhibitory on older adult male mating behavior[c]

[a] *Martin-Sanchez* et al. *(2015).*
[b] *Roberts* et al. *(2012).*
[c] *Ferrero* et al. *(2013).*

higher mutation rates compared to other mammals, and a wide range of developmental flexibility based on early life experience, which all contribute to their remarkably successful environmental adaptability. The deme is composed of a dominant breeding male, a hierarchy of females, subordinate males, and juveniles. Wild mice occupy territories measuring just a few square meters when food is abundant to several square kilometers. Mice are crepuscular (active during the twilight hours of dawn and dusk), strongly territorial, and omnivorous. Coprophagy contributes to approximately one-third of their ingesta as an essential nutritional activity. Aside from territoriality, social interactions, breeding, burrowing (when conducive substrates are available), and nest building are major activities. In managing laboratory mice, it is important to understand the complex behavioral biology of their free-living counterparts (Latham and Mason, 2004).

Chemo-olfactory communication is mediated through extremely diverse chemical factors that trigger innate (non-learned) social responses among conspecifics, known as *pheromones* (Table 3.10). Pheromones have been traditionally divided into two broad categories: *releaser* pheromones, which elicit an immediate behavioral response, and *primer* pheromones, which mediate a slowly developing and longer-lasting endocrine response. This original definition of pheromone categories has been expanded to another category, termed *signaler* pheromones, which convey individual or group identity, as well as mediating parent–offspring recognition and mate choice. The biology and genetics of pheromone signaling is being extensively studied in the mouse as a model of mammalian pheromone communication (Brennan and Zufall, 2006; Rodriguez and Boehm, 2009).

Mouse pheromones are excreted in the urine, as well as plantar, salivary, lacrimal, preputial, and mammary glands. In the urine, *major urinary proteins* (Mups), small peptides, MHC class I peptides, volatile chemicals, and sex hormones all contribute to chemosignals that communicate dominance, kinship, diversity, and gender. Wild mice possess a great deal of individual variations of

these elements, providing a 'bar code' that distinguishes individuals. Inbreeding of laboratory mice has reduced individual variation, but each inbred strain possesses a characteristic array of signals, and to a certain extent, unique signals exist among individuals within a strain (Sharrow *et al.*, 2001; Sturm *et al.*, 2013). Pheromones are detected by sensory neurons in the vomeronasal organ, the olfactory epithelium, and the lesser known septal organ of Masera within the olfactory epithelium, and the Gruenberg ganglion, which is located at the anterior end of the nasal cavity (Breer *et al.*, 2006; Chamero *et al.*, 2012; Liberles and Buck, 2006; Restrepo *et al.*, 2004). Neuronal signals are transmitted to the ganglion layer of the olfactory bulb, and thence to the brain.

Mups are important components of chemosensory communication in mice, and also an important occupational hazard to human handlers. Chromosome 4 contains a cluster of 21 Mup genes, plus a number of pseudogenes. Mups are small soluble proteins known as lipocalins, which bind small organic chemicals (pheromones) with high affinity, and function as pheromone transporters and stabilizers (thereby contributing to slow release), but also act as protein pheromones themselves. They are synthesized in the liver and excreted in the urine, as well as nasal mucosa, lacrimal glands, and salivary glands. Their endogenous role on metabolic activity is not yet understood. Male mice excrete significantly more Mups in the urine than females. One well-characterized Mup is 'darcin', named after Fitzwilliam Darcy, the romantic hero in Pride and Prejudice. As its name implies, it is a female attractant. Mups also act as *kairomones*, which function as chemical signals between species. For example, cat and rat Mups invoke fear in mice. Mups are important in the laboratory animal management context, as they are excreted in copious amounts (1–5 mg/ml in urine) and are potent allergens for humans, particularly Mus m 1 (Ag1 or MA1), which is encoded by the Mup 17 gene (Sharrow *et al.*, 2001).

Chemosensory communication has numerous behavioral effects that influence mouse social interactions. One of the most studied behavioral effects is the *Bruce effect*, or pregnancy block, which is a complex physiologic response in which recently conceived females resorb fetuses during early pregnancy in the presence of an unrelated male, particularly a dominant male. The continued presence of the original mate protects the female from this effect (Bruce, 1959). The *Vandenbergh effect* results in acceleration of puberty of juvenile females in response to male urine (Vandenbergh, 1973). The *Lee–Boot effect* occurs among group-housed females that are isolated from males, in which there is suppression of estrus cyclicity (Van Der Lee and Boot, 1955). The *Whitten effect* results in synchronization of estrus among a group of females in response to a male (Whitten *et al.*, 1968). The Lee–Boot and Whitten effects are utilized in the laboratory to assist in induction of synchronized timed pregnancy, but the Bruce effect can have deleterious consequences on breeding colonies when foreign males are introduced to a breeding colony, as pheromone communication can occur in the absence of direct contact.

The above effects are well-defined pheromone-driven behavioral responses, but chemosensory communication has a myriad of other effects. Estrus, pregnant, or lactating females also accelerate puberty among juvenile females. Females use odor cues to avoid parasite-laden males, males prefer odors of estrus females, and estrus females prefer odors of dominant males. Mice have strong mating and social preferences based upon MHC proteins, which indicate genetic relatedness. Maternal recognition of young is also MHC-related, and pups prefer nest odors of maternal and sibling pups based upon MHC relatedness. Male aggression against unrelated males is also a strong MHC-related phenomenon. MHC haplotypes determine not only MHC proteins in the urine, and MHC-specific olfactory receptors, but also the composition of volatile chemicals in the urine (Kelliher and Wersinger, 2009).

The complexity of social communication extends to auditory stimuli as well. Male mice utilize ultrasonic 'birdsong' to vocally communicate and attract females. Mouse vocalization patterns are largely genetically innate and unique to each strain of mouse, but they can also be modified, or learned, to a limited extent (Arriaga *et al.*, 2012).

The behavioral biology of the mouse is highly complex, and depends upon genetic, physiologic, social, and environmental variables, which all impact on how laboratory mice can best be managed in captivity. It is clear that this rich complexity cannot be fully addressed under laboratory conditions, but that does not mean that basic needs, such as nest building, burrowing, foraging, and olfactory environments, cannot be provided. For example, intermale aggression, which is particularly apparent in some strains of mice such as BALB/c and Swiss-origin stocks and strains, can be minimized by maintaining males from infancy as sibling groups, since adult siblings tend not be aggressive to one another. This sibling bond, however, can be easily broken by short-term separation. Environmental enrichment often features provision of plastic houses, which may make vivarium managers feel good, but maximal enrichment can be provided by provision of nesting material, which includes structural scaffolding, such as crinkled cardboard, which facilitates construction of three-dimensional nests. Mouse nests are replete with 'appeasement' pheromones, thereby contributing to harmony within the cage, whereas introduction of dirty bedding has the opposite effect. Frequent cage changing, including removal of established nests, is highly stressful and disruptive to social harmony within a cage. Provision of appropriate and adequate amounts of bedding material that is conducive to burrowing is desirable. It is important to remember that mice are

socially gregarious, and that mouse welfare is optimally enriched by other mice within a socially harmonious deme (Latham and Mason, 2004; Van Loo *et al.*, 2003).

A laboratory mouse ethogram, defined as an operationalized list of mouse behaviors, arranged by their adaptive meaning to the animal, is available on the web: www.mousebehavior.org. Behavioral phenotyping, particularly of transgenic mice, is used extensively in genomic research. A wide variety of standardized test batteries and approaches are used, depending upon the focus of research (reviewed in Crawley 2008). Initial behavioral evaluations include general health, body weight, body temperature, appearance of the fur and whiskers, and neurological reflexes assessment. Specific tests include observations of home cage behaviors, 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.

Novel and complex environmental enrichment in animal housing conditions facilitates enhanced sensory and cognitive stimulation as well as physical activity. Environmental enrichment and exercise have beneficial effects such as cognitive enhancement, delayed disease onset, enhanced cellular plasticity, and associated molecular processes in animal models of brain disorders (Pang and Hannan, 2013).

D. Immunology

The immune system of the mouse is very similar to that of humans. The availability of inbred mouse strains, in which each individual animal expresses identical MHC alleles so that tissues and cells can be transplanted without tissue rejection, greatly simplifies and indeed enables functional analyses of immune system components not possible with any other outbred mammalian species. In addition, the ability to genetically manipulate the mouse genome, adding to, altering, and deleting existing genes, enables unprecedented *in vivo* analysis of immune cell functions. It is for these reasons that the mouse is the primary animal model for immunology research.

1. Tissues of the Immune System

The immune system is an unusual organ system in that it consists of both solid tissues and various migrating cell populations. The bone marrow and thymus are considered primary lymphoid organs, as sites of hematopoiesis and B- and T-lymphocyte development, respectively. Lymph nodes, spleen, and intestinal Peyer's patches are considered secondary lymphoid tissues, as sites of immune response initiation. Lymph nodes and spleen are analyzed frequently for studies of immune responses and as organs for immune cell isolation. Tertiary lymphoid tissue sites are those that form in other solid organs in response to an insult or microbial exposure. Among them are the lymphoid cell aggregates of the gastrointestinal and respiratory tract, also called 'gut-associated lymphoid tissue' (GALT) and bronchus-associated lymphoid tissues (BALT).

2. Cells of the Immune System

Leukocytes are classified as belonging to the innate or adaptive immune system. The innate immune system responds rapidly to an antigen insult via recognition of pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide, bacterial flagellin, single (s)- and double-stranded (ds) RNA, and non-methylated DNA, via extracellular or intracellular pattern recognition receptors (PRRs). Receptors include the Toll-like receptors (TLRs), such as TLR4 (recognizing LPS), TLR3/7 (ss and dsRNA) and TLR9 (DNA), NOD-like receptors (NOD1/2), and RIG-like receptors (RIG-I, MDA-5) among others (Takeuchi and Akira, 2010). Cells of the innate immune system are monocytes/macrophages, granulocytes and dendritic cells as well as innate-like lymphocyte populations (ILC) 1, 2 and 3, which include natural killer (NK) cells (Spits *et al.*, 2013). Cells of the adaptive immune system (T and B lymphocytes) express a highly antigen-specific receptor that has arisen through gene rearrangement (T-cell and B-cell receptors, respectively). B cells of the B-1 lineage and γδ T cells are regarded as innate-like cells, as they express a rearranged antigen receptor but seem to respond in an innate-like manner.

Leukocytes are identified and classified by sets of monoclonal antibodies (mAb) against uniquely expressed surface receptors, typically measured by flow cytometry. Identification of a unique receptor by one or more mAb of the same specificity leads to the assignment of a receptor name, as a 'cluster of differentiation (CD)'. For example, T cells are differentiated into two subsets based on their expression of either CD4 or CD8. $CD4^+$ T cells (T helper cells) recognize peptides presented in MHC class II and promote B-lymphocyte activation and activate and regulate cellular immune responses via secretion of differing cytokines (see below). $CD8^+$ T cells recognize antigenic peptides presented in MHC class I and serve as cytotoxic cells during the cell-mediated immune response where they can destroy infected cells (e.g., against cells containing infectious agents).

3. Secreted Products of the Immune System

Immunoglobulins

The major function of B cells is to respond to an encounter with an antigen/pathogen with the production of highly antigen-specific immunoglobulins (Ig; antibodies), which can bind to and inactivate pathogens and toxins. Activation of B cells can lead to their differentiation to plasma cells, which produce large amounts of Ig. Five

classes or Ig 'isotypes' can be distinguished, which differ in effector function: IgM, IgG, IgA, IgE, and IgD. The latter is expressed only on the surface of B cells in mice. The IgG class, the most abundant antibody class in the serum, is further divided into subtypes: IgG_1, $IgG_{2a/c}$, IgG_{2b}, and IgG_3. Polymorphisms exist on the Ig locus such that some strains of mice produce the IgG2a subtype (e.g., BALB/c), whereas others produce IgG2c (e.g., C57BL/6) (Zhang *et al.*, 2012). Additional allelic polymorphisms of the locus also exist. For example, BALB/c and 129SV mice express the Igh-a allotype, whereas C57BL/6 mice express the Igh-b allotype. Recombinant inbred strains of mice exist for both BALB/c and C57BL/6, which harbor the reciprocal Igh locus (i.e., Igh-b for BALB/c and Igh-a for C57BL/6 mice). These mice are useful tools for tracking B cells following adaptive cell transfer via allotype-specific mAb (see below).

Immunoglobulin isotype production varies according to the type of immunogen used to evoke the response. IgM is secreted short term after initial exposure to an antigen, followed by the other Ig isotypes. In viral and intracellular bacterial infections, $IgG_{2a/c}$ is dominant, whereas in extracellular bacterial infections IgG_1 dominates the response. IgG_{2b} and IgG_3 are usually induced to carbohydrate or lipid antigens. IgE is linked to parasitic infections and to allergy. Serum antibodies specific for an immunogen can often be measured for the life of the animal. While serum IgA levels are low, IgA is the highest produced Ig in mice. IgA production, however, occurs in plasma cells lodged in the lamina propria of mucosal tissues, from where the IgA is actively transported in dimeric form onto the luminal surface of mucosal tissues as 'secretory' IgA (Brandtzaeg, 2009).

Cytokines and Chemokines

Cytokines are secreted signaling molecules involved in cell–cell communication in a complex biological system (Table 3.11). These include the large family of interleukins (ILs, currently IL-1 to IL-37), tumor necrosis factors (TNFs), interferons (Type I, II, and III) and growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and stem cell factor (SCF). Cytokine secretion often occurs in response to recognition of antigen via PRR or TCR. Because of their importance in modulating immunity to antigenic stimuli, mice with specific deletions or overexpression of individual cytokines have been made and have contributed to a detailed understanding of many of their often pleotropic functions (Akdis *et al.*, 2011).

Chemokines are a similarly large group of small, secreted molecules that regulate cell trafficking to sites of antigen encounter but also facilitate cell–cell contact by acting as chemoattractants. Chemokines are grouped according to the number of cysteines and disulfide bonds in the molecule into C-X-C-, C-C, C, and CX_3Cl Chemokine ligands (L) and receptors (R) and designated accordingly as CXCR1-7/CXCL1-16 and CCR1-10/CCL 1-28 (Allen *et al.*, 2007).

4. Polarization of the Immune Response

Immune responses must be coordinated to provide the most appropriate effector functions for the type of pathogen/antigen encountered. Immune effector responses differ depending on the life cycle (facultative or obligate intracellular, extracellular, localized, systemic, etc.) and antigen types displayed by the encountered antigen/pathogen, because this affects the type of PRR engaged and activated. PRR engagement leads to cytokine and chemokine responses by the first responders, i.e., epithelial cells, local macrophage populations and other innate cells. The type of cytokines and chemokines produced then dictates the types of cells recruited to the site of infection and their subsequent differentiation and functions. The PRR engagement also leads to antigen uptake, activation and migration of dendritic cells (DCs) from the site of insult to the regional lymph nodes, where DCs present antigen peptides on MHC molecules to T cells. In addition, the DCs secrete cytokines induced by the initial PRR activation, which cause the differentiation of CD4 T cells towards a particular effector response. For example, secretion of IL-12 in response to activation of TLR2 or 4 will result in the induction of interferon-gamma (IFN-γ) production by CD4 T cells, whereas IL-6 and TGF-β production by DC will induce CD4 T cells to secrete IL-17 (Kara *et al.*, 2014). Because the DC translates signals from PRR at the site of infection into differentiation signals for T cells in the lymph tissues, these cells are regarded as a 'bridge' between the innate and adaptive immune systems.

The specific Ig isotype secreted in response to a pathogen depends to a large degree on the type of cytokine produced by CD4 T cells that provide 'T-cell help' for B cells. T cells that interact with B cells are identified as a discrete subset termed 'T follicular helper cells (T_{FH})' and it is their cytokine profile that directs B cells to secrete a particular Ig isotype (Kara *et al.*, 2014). The classic T_H1/T_H2 dichotomy outlined above was in part shaped by the observation that IFN-γ production will lead to switching of B cells to secrete IgG2a/c, whereas production of IL-4 leads to the secretion of IgG1.

Interestingly, it appears that the cytokine profile induced by the effector T-cell population is mirrored by the innate immune response. Innate-like lymphocytes also have effector phenotypes that correspond to those of CD4 T cells and are induced by the same signals and transcriptional regulators (Spits *et al.*, 2013) and the same appears to be true also for macrophages and other innate immune cells (Sica and Mantovani, 2012). While initial studies identified two particular antagonistic effector response types (termed T_H1 and T_H2 and classified by

TABLE 3.11 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-CSFs, granulocyte-macrophage colony-stimulating factors; NK, natural killer; TNF, tumor necrosis factor; TGF, tumor growth factor.*

T-cell production of IFN-γ and IL-4, respectively), more recent studies now demonstrate a much wider array of effector responses in which innate and adaptive immunity acts together to reinforce an immune response phenotype as well as modulate its size by induction of T regulatory cells (T_{regs}) that generate inhibitory cytokines (Kara *et al.*, 2014; Sica and Mantovani, 2012; Spits *et al.*, 2013). The use of cytokine-deficient and reporter mice that enabled the identification of cytokine-producing cells via expression of a fluorescent reporter was particularly valuable for the development of this more nuanced view of the quality of immune responses.

5. Experimental Approaches to Study Immune System Components

Spontaneous mouse models of immune deficiencies have been used extensively in research. Their use, plus the expanding number of knockout, transgenic, and dominant negative mouse mutants, has advanced understanding of human immune deficiency diseases as well as basic understanding of the immune system (Table 3.12). Interbreeding of multiple immune-deficient mice has allowed the development of 'humanized' mice in which immune cells of the mouse are replaced with those of humans. While many challenges remain to fully

TABLE 3.12 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
NOD/SCID/IL-2Rγ$^{-/-}$	Multiple immunodeficiencies derived from NOD and SCID mice with loss of IL-2 receptor gamma chain[1]	No T-, B-, or NK-cell activity Decreased complement Impaired macrophage and dendritic cell functions	Model for efficient engraftment of human lymphoid cells
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
1pr and gld mice	Impaired apoptosis from Fas (1pr) or Fas ligand (gld) defect	Generalized lymphoproliferative disease (gld), autoimmunity, immunodeficiency	Immune deficiencies 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

replenish mice with components of the human immune system, the use of immune-deficient NOD/severe combined immunodeficient (SCID)/IL-2R$\gamma^{-/-}$ recipients for transfer of human peripheral blood lymphocytes, cord-blood or bone marrow-derived CD34+ stem cells with human liver and thymus (BLT-mice) is yielding promising results (Akkina, 2013).

Investigators using genetically engineered mice are constantly reminded 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.

Experimental approaches are being increasingly used to refine the knockout technology by restricting a specific genetic deficiency to a particular tissue of interest using the Cre-lox system, in which tissue-specific or temporal restricted expression of the Cre recombinase induces the deletion of a 'floxed' gene (Mak *et al.*, 2001). Transgenic mice are available that restrict Cre expression to various hematopoietic cells or tissue or drive Cre recombination following injection of tamoxifen. Other approaches are the generation of 'bone marrow irradiation' chimeras. Here, inbred wild-type mice or mice deficient in certain immune cells (Table 3.12) are lethally irradiated by exposure to a gamma-irradiation source to deplete the hematopoietic stem cells. These are then replaced by transfer of bone marrow cells to the irradiated mice. Reconstitution of the hematopoietic system is usually achieved within about 6 weeks, during which time mice are provided with antibiotic-containing drinking water to avoid infections of these temporarily immune-compromised animals. Transfer of bone marrow from a congenic knockout restricts the genetic defect to the hematopoietic system.

A mix of bone marrow from two sources is also often used to generate tissue-specific knockouts. For example, mixing a bone marrow from T-cell-deficient mice (75%) with that of a gene knockout (25%) generates 'mixed bone marrow chimeras' in which all T cells only develop from the knockout, thus lack the gene of interest, whereas most of the other cells T from the wild-type source, effectively constraining the genetic defect to the T-cell population. Sets of congenic mice with defined allotypic differences are often used to confirm the source of individual cells. Such markers include the gene locus CD45.1/CDC45.2 or CD90.1/CD90.2 (Thy1.1/Thy1.2). Alternatively, cells may express a fluorescent transgene, such as green-fluorescent protein (GFP). Identification is usually performed by flow cytometry, or less commonly by immunofluorescence or immunohistochemistry. Generation of bone marrow chimeras circumvents the time-consuming breeding of Cre recombinase-expressing flx/flx mice. However, numerous controls are needed to exclude off-target effects due to irradiation damage.

Repeat injection of antibodies targeting specific cell populations is another rapid approach that avoids the potential for irradiation damage and allows short-term depletion of individual cell subsets. Its main disadvantage is the need to identify mAb that bind to surface receptors uniquely expressed by a cell subset of interest and the verification of the efficacy of the depletion. Frequently used is antibody treatment for the short-term depletion of T-cell subsets using mAb against CD4 or CD8 as well as individual cytokines.

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

1. Microbiological Surveillance and Diagnostics

Many microbial agents have the potential to cause disease in mice or 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. Surveillance should encompass resident mice *and* mouse products (serum, cell lines, transplantable tumors) procured from external sources. Because surveillance strategies will vary with research needs and operating conditions, it is prudent to consult a number of sources, such as the Federation of European Laboratory Animal Science Associations (FELASA) (Nicklas *et al.*, 2002) and commercial laboratories, for guidance. Detailed discussion of microbial quality control is provided in Chapter 10. There are also recommendations regarding specific agents in following sections. Diagnostic methods involve gross and microscopic pathology, parasitology, microbial isolation

and culture, serology, and PCR. Serology is particularly important for viral surveillance, and now relies principally on enzyme-linked immunosorbent assay (ELISA), multiplex fluorescent immunoassay (MFI) for simultaneous detection of antibodies to multiple agents (Hsu *et al.*, 2007), indirect fluorescent antibody (IFA) assay, or hemagglutination inhibition (HAI), with the latter two methods generally used for confirmation (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). Mouse antibody production (MAP) testing has been historically used for testing biological materials for contamination by infectious agents. PCR panels for murine infectious agents are now commercially available and have cost and time-saving advantages as well as improved assay sensitivity and specificity. Beyond the classic bacterial and viral murine infections, PCR assays are now available for endo- and ectoparasites (see Chapter 11).

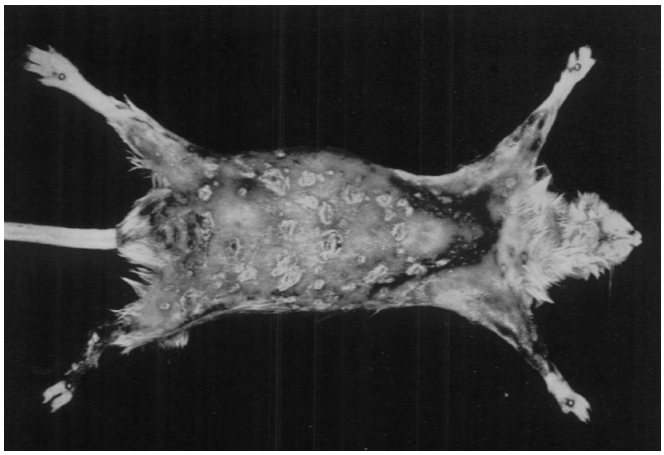

FIGURE 3.12 Mouse after depilation to reveal the rash associated with mouse pox. *From Fenner (1948a).*

2. *Viral Diseases*

a. Mousepox (Buller and Fenner, 2007; Esteban and Buller, 2005; Fenner, 1948b, 1949a, b)

Etiology Mousepox is caused by *Ectromelia virus* (ECTV), an orthopoxvirus that is antigenically and genetically closely related to a number of other poxviruses, including vaccinia, variola, and cowpox viruses. The original isolate of ECTV, known as the Hampstead strain, was discovered by J. Marchal in 1930 (Marchal, 1930) as the cause of epizootic disease among laboratory mice in England. The disease featured amputation of extremities, which Marchal termed *ectromelia* (from the Greek, *ectro*, amputation and *melia*, limb). Other strains of the virus include Moscow, NIH-79, Washington University, St. Louis 69, Beijing 70, Ishibahsi I–III, and Naval (NAV) strains, which vary in virulence, but are essentially indistinguishable genetically and serologically, suggesting a common origin. Virus can be isolated from infected tissues by inoculation of cell cultures (BS-C-1, HeLa, L cells) or embryonated eggs. The natural host (and original source of infection of laboratory mice) of ECTV remains unknown.

Clinical Signs The expression of clinical signs reflects an interplay among virus-related factors, including virus strain, dose and portal of entry, and host-related factors, including age, genotype, immunological competence, and gender (Brownstein *et al.*, 1991a). During natural epizootics, it was observed that A, BC, DBA/1, DBA/2, and CBA strains developed acute fatal infections, whereas C57BL/6 mice were resistant to severe disease (Briody, 1966). Experimental studies have shown that all strains of mice are susceptible to infection, but BALB/c, A, DBA/2, and C3H/He mice were highly susceptible, AKR and SJL mice were moderately susceptible, and C57BL/6 mice were highly resistant to lethal infection (Bhatt and Jacoby, 1987c; Wallace and Buller, 1985). 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 NK cells (Brownstein *et al.*, 1991a; Jacoby *et al.*, 1989). The nuances of cytokine and cellular immune responses to ECTV infection have received recent attention (reviewed in Buller and Fenner (2007) and Esteban and Buller (2005)). Outbreaks among susceptible mice are often volatile, with variable morbidity and high mortality in susceptible strains of mice. Clinical signs such as ruffled fur or prostration may occur for only a few hours before death. Mice that survive acute infection may develop chronic disease characterized by a focal or generalized rash anywhere on the body (Fig. 3.12). Conjunctivitis also may occur. Skin lesions usually recede within several weeks, but hairless scars may 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 recent outbreaks have been 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 direct contact and skin abrasions. Cage-to-cage transmission is low and can be virtually nil if filter-topped cages are used (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, 1987d). Aerogenic exposure is not a major factor in natural outbreaks, and arthropod-borne transmission does not appear to occur. Virus-free progeny can be obtained

from immune dams (Bhatt and Jacoby, 1987b). However, intrauterine infection and fetal deaths, albeit rare, have been reported.

Natural transmission is facilitated by intermediately resistant mice, which survive long enough to develop skin lesions that can shed virus for relatively long periods of time. 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 subclinical infections. However, infections in resistant mice tend to be short-lived. Highly susceptible mice are a relatively small hazard for dissemination of infection, if properly discarded, because they die before virus shedding becomes prominent. Thus, juxtaposition of resistant or intermediately resistant infected mice with 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 The classic descriptions of ECTV pathogenesis by Fenner remain timely, including the frequently cited and reproduced figure summarizing the pathogenesis of infection (Fig. 3.13) (Fenner, 1948b). Interest in smallpox has renewed the interest in ECTV as a model of host response to infection (Esteban and Buller, 2005). ECTV multiplies in the cell cytoplasm and produces two types of inclusion bodies. The A type (Marchal body) is well demarcated and acidophilic in histological sections. It is found primarily in epithelial cells of skin (Fig. 3.14) or mucous membranes and can also be found in intestinal mucosa. 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. ECTV antigen can be readily visualized by immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections (Esteban and Buller, 2005; Jacoby and Bhatt, 1987).

Following skin invasion, viral multiplication occurs in the draining lymph node and a primary viremia ensues. 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

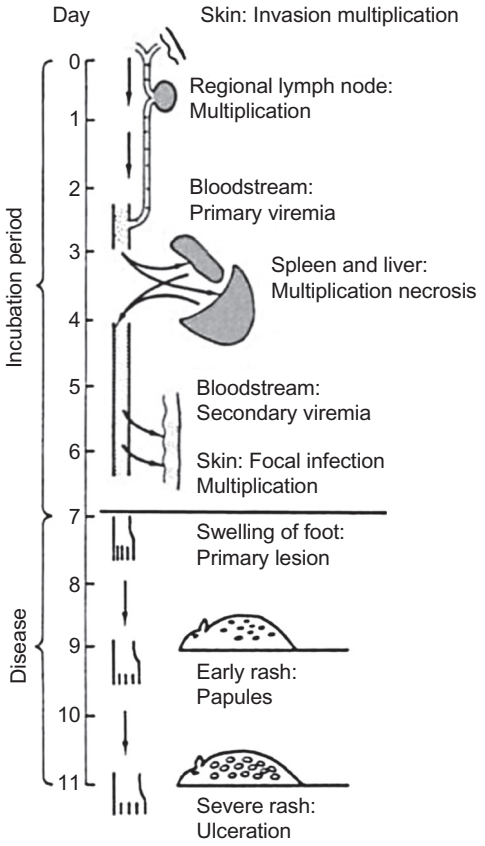

FIGURE 3.13 Diagram illustrating the pathogenesis of mousepox. *From Fenner (1948b).*

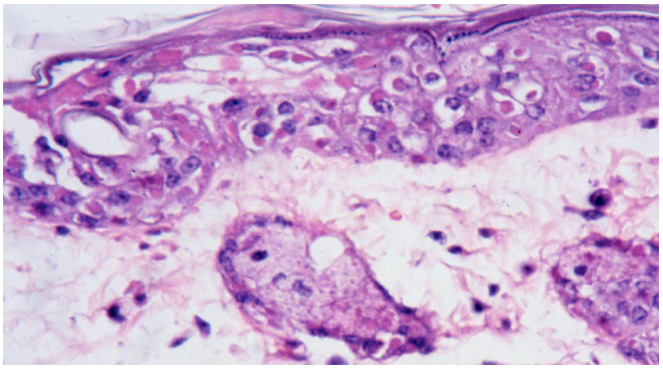

FIGURE 3.14 Skin from a mouse infected with ectromelia virus. Note intracytoplasmic inclusions. *Courtesy of S.W. Barthold.*

indicative of necrosis develop throughout the liver (Fig. 3.15). 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. Necrosis and scarring of red and white pulp can produce a macroscopic 'mosaic' pattern of white and red–brown (Fig. 3.16). Necrosis of thymus, lymph nodes, Peyer's

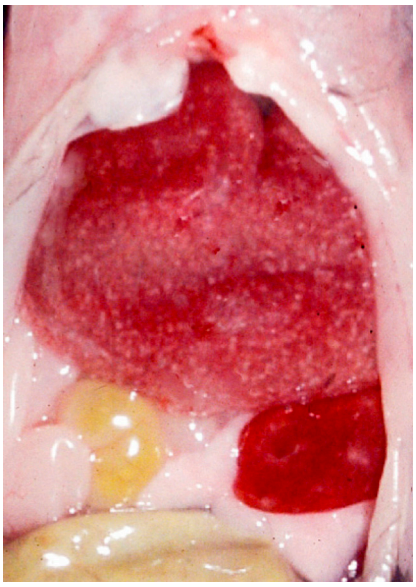

FIGURE 3.15 Multifocal necrotizing hepatitis and splenitis in a mouse during the acute phase of a natural infection with ectromelia virus. *From Percy and Barthold (2007), with permission from Nina Hahn.*

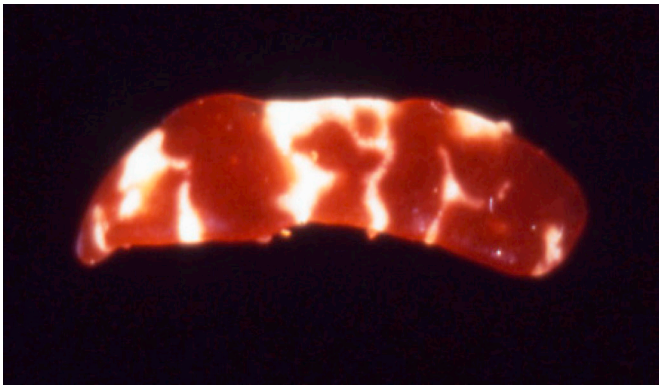

FIGURE 3.16 'Mosaic spleen' from a mouse that survived acute mousepox. The pale bands are due to fibrotic scarring following severe necrosis.

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

Diagnosis Mousepox can be diagnosed from clinical signs, lesions, serological tests, and demonstration of virus or viral antigen in tissues. Observation of characteristic intracytoplasmic eosinophilic inclusions aids detection of infection. Several serological tests are available to detect mousepox. Historically, the standard test was HAI, using vaccinia antigen as a source of hemagglutinin. ELISA is more sensitive and specific and has replaced HAI for serological monitoring among nonvaccinated mice (Buller *et al.*, 1983). ECTV infection also can be detected by IFA (Buller *et al.*, 1983) and PCR. 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 Sendai pneumonia, mouse hepatitis, and Tyzzer's disease. The latter two 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, ascariasis, 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 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 observed for clinical signs and tested for seroconversion after several weeks. Depopulation and disinfection must be carried out 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 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,

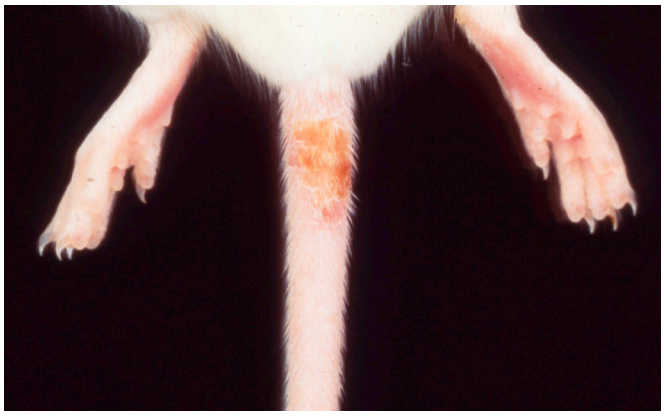

FIGURE 3.17 Vaccination 'take' in a mouse vaccinated by scarification of the tail base with the IHD-T vaccinia virus. *From Jacoby et al.* (1983).

with the caveat that progeny will be transiently seropositive with maternally derived antibody.

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, 1987a) (Fig. 3.17). 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 (Shellam, 2007, 96)

Mice are naturally susceptible to two herpesviruses from the subfamily Betaherpesvirinae and in the genus *Muromegalovirus*, the two species *Murid herpesvirus 1* (of which one of the members is mouse cytomegalovirus (MCMV)) and *Murid herpesvirus 3* (of which one of the members is mouse thymic virus (MTV)). They are species-specific viruses and distinct from each other and from other rodent herpesviruses. MCTV has received considerable attention as a model of human CMV infection.

MOUSE CYTOMEGALOVIRUS INFECTION

Etiology Mouse cytomegalovirus (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. Cocultivation may be required to rescue latent virus.

Clinical Signs MCMV causes subclinical infection in adult immunocompetent mice, but experimental inoculation of neonates can cause lethal disease due to multisystemic necrosis and inflammation.

Epizootiology The prevalence of MCMV in laboratory mice is probably uncommon but undefined, since infection is clinically silent and serological surveillance is not widely practiced. Wild mice are commonly infected and serve as a natural reservoir for infection, which implies that the entry of virus into a modern vivarium is most likely to occur from contaminated animal products.

Persistence is a central feature of nonlethal infection. Persistently infected mice 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. 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. 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 antibody titers are low during acute infection and difficult to find during chronic infection. Serology and PCR-based diagnosis are available, but neither is widely used because of assumptions that infection has a very low prevalence. Detection of enlarged cells with intranuclear inclusions, especially in salivary glands, is 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 MTV. The latter virus can produce necrosis of thymic and peripheral lymphoid tissue when infant mice are experimentally inoculated. 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 mouse polyoma virus. Like MCMV, MTV infects the salivary gland as its primary target organ.

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 MCMV 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.*

MOUSE THYMIC VIRUS INFECTION (Morse, 1989)

Etiology Mouse thymic virus (MTV) is a herpesvirus (murid herpesvirus 3) 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 subclinical.

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. 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 and lymphoid tissue with tropism for $CD4^+$ T cells in mice inoculated within approximately 1 week after birth. 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 inclusions. Necrosis is followed by granulomatous inflammation and syncytium formation. Reconstitution of lymphoid organs takes 3–8 weeks.

Diagnosis Thymic necrosis associated with intranuclear viral inclusions is the hallmark lesion. Viral antigen can be detected by immunohistochemistry. Serologic detection is effective, but generally not utilized, and is potentially negative in neonatally exposed mice. 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 thymic necrosis 10–14 days later. PCR 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, 2007; Besselsen *et al.*, 2006)

Parvoviruses are among the most common viral infections in contemporary laboratory mouse populations (Livingston *et al.*, 2002) (Pritchett-Corning *et al.*, 2009), and pose major challenges to both detection and control. The mouse parvoviruses are composed of two antigenically and genetically distinct but related groups, including minute virus of mice (MVM) and mouse parvovirus (MPV), with each group containing a number of strains. The International Committee on Taxonomy of Viruses classifies MPV, MVM, and several other rodent parvoviruses into one genus, *Protoparvovirus*, and species, *Rodent protoparvovirus 1*, but these viruses will be treated separately herein.

MINUTE VIRUS OF MICE

Etiology Minute virus of mice (MVM) is a small (5-kb) single-stranded DNA virus. The prototypic strain is designated MVMp. An allotropic variant with immunosuppressive properties *in vitro* is named MVMi, and additional

named strains include MVMc and MVMm. The genome encodes two nonstructural proteins, NS-1 and NS-2, which are highly conserved among the rodent parvoviruses and account for prominent cross-reactivity in serological assays that utilize whole virus antigen. The viral capsid proteins, VP-1 and VP-2, are virus-specific and form the basis for serological differentiation of MVM from 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 MVM infections are subclinical. Neonatal mice of some inbred strains are experimentally susceptible to lethal renal and/or intestinal hemorrhage during MVMi infection, but this syndrome has not been reported in natural outbreaks. Experimental inoculation of adult C.B-17-*Prkdc*^{scid} (SCID) mice with MVMi results in lethal infection (Lamana *et al.*, 2001; Segovia *et al.*, 1999), and similar severe illness has been noted in naturally infected B-cell-deficient NOD. Cg-*H2H4-Igh6* null mice (Naugler *et al.*, 2001).

Epizootiology MVM is a common virus that naturally infects laboratory mice, but appears to be less common than MPV (Besselsen *et al.*, 2006; Livingston *et al.*, 2002).

MVM is moderately contagious for mice, its only known natural host. Virus can infect the gastrointestinal tract 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, 1983; Smith and Paturzo, 1988). Infection appears to last less than 1 month, even in oronasally inoculated neonatal mice, but immunodeficient mice may be persistently infected. There is no evidence that MVM is transmitted *in utero.*

Pathology Natural infections or experimental inoculation of adult mice appears to be nonpathogenic. 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 MVMi can cause renal hemorrhage and infarction (Brownstein *et al.*, 1991b). 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. Infection of immunodeficient mice, including SCID and B-cell-deficient mice, results in lethal damage to granulomacrophagic, megakaryocytic, and erythrocytic hematopoietic tissue with severe leukopenia (Lamana *et al.*, 2001; Naugler *et al.*, 2001; Segovia *et al.*, 1999). Intranuclear viral inclusions and viral antigen have been observed in splenic mononuclear cells of B-cell deficient mice (Naugler *et al.*, 2001).

Diagnosis Serology is the primary method of detecting infection, which utilizes recombinant MVM and MPV major capsid viral proteins (VP2) as antigens, which discriminate between the two groups of mouse parvoviruses. In contrast, the conserved nonstructural protein, NS1 can be used to detect antibody to both groups, but is less sensitive than VP2 assays (Livingston *et al.*, 2002). MVM infection also can be detected by PCR, *in situ* hybridization, and immunohistochemistry. PCR assays can be used to detect MVM- or MPV-specific VP2 or all rodent parvovirus group specific NS1 exons (Besselsen, 1998; Besselsen *et al.*, 2006). MVM can be isolated from the 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. MPV has been shown to be successfully eliminated by a cage-by-cage test (serology and fecal PCR) and cull approach, although there are no published reports confirming the success of this strategy for eliminating MVM (Macy *et al.*, 2011). Cesarean rederivation or embryo transfer may also be used to rederive virus-free progeny. 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 can occur; therefore, infection can be introduced to a colony through inoculation of contaminated cell lines. Failure 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. MVMi has the potential to inhibit the generation of cytotoxic T cells in mixed lymphocyte cultures.

MOUSE PARVOVIRUS

Etiology Mouse parvovirus (MPV) is among the more common viruses detected within contemporary

mouse colonies, and is more common than MVM (Livingston *et al.*, 2002; Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). MPV was initially 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) (McKisic *et al.*, 1993). Molecular analysis of MPV indicates that regions encoding the NS proteins are similar to those of MVM (and other rodent parvoviruses). However, they differ significantly in regions encoding the capsid proteins, accounting for their antigenic specificity. 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). Immortalized T cells (L3) are the only cells found thus far to support replication of MPV. There are three genetically distinct variants of MPV, including MPV-1, MPV-2, and MPV-3. MPV-1 includes a number of closely related variants, including MPV-1a, MPV-1b, and MPV-1c. In addition, a hamster parvovirus isolate is closely related to MPV-3, which is infectious to mice and likely to be of mouse origin (Besselsen *et al.*, 2006; Christie *et al.*, 2010).

Clinical Signs MPV infection is clinically silent in infant mice and adult immunocompetent or immunodeficient mice (Besselsen *et al.*, 2007). Immunologic perturbations are the most likely signs of infection (McKisic *et al.*, 1993).

Epizootiology MPV causes persistent infection in infant and adult mice, a property that differentiates it from MVM. *In situ* hybridization has identified the small intestine as a site of viral entry and early replication, but respiratory infection cannot be excluded. Experimental studies following inoculation of neonatal BALB/c and C.B-17-*Prkdc*^{scid} (SCID) mice revealed that BALB/c mice shed high levels of virus for 3 weeks, with transmission to sentinels exposed during the first 2 weeks of infection. Thereafter, BALB/c mice shed extremely low virus intermittently. In contrast, SCID mice shed high levels of virus until weaning, but lower levels at 6 weeks of age, yet they effectively transmitted infection to sentinels at all stages of infection (Besselsen *et al.*, 2007). Others have shown that transmission of MPV by Sencar mice inoculated as infants was intermittent up to 6 weeks, whereas transmission by mice inoculated as weanlings occurred during the first 2 weeks of infection (Smith *et al.*, 1993). Transmission to BALB/c progeny from infected dams was shown to occur, but embryo transfer rederivation was found to be successful in experimentally infected SCID mice (Besselsen *et al.*, 2008). 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).

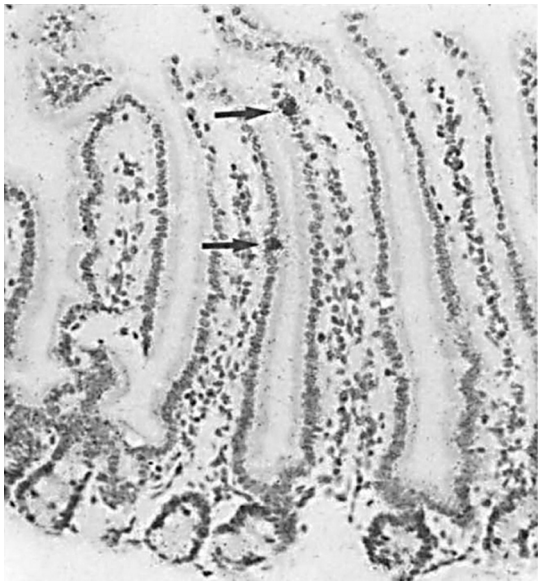

FIGURE 3.18 Mouse parvovirus (arrows) in the intestine after oronasal inoculation of an adult mouse. *In situ* hybridization.

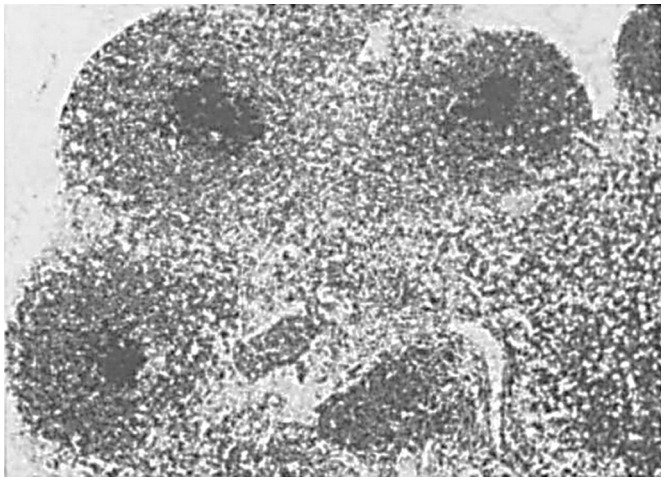

FIGURE 3.19 Mouse parvovirus in the mesenteric lymph node of a persistently infected mouse. *In situ* hybridization localizes the virus to the germinal centers.

Pathology MPV appears to enter through the intestinal mucosa, which is a site of early virus replication (Fig. 3.18). Acute infection is widespread but mild, involving the lung, kidney, liver, and lymphoid organs. Histological lesions are not discernible. 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. 3.19).

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. Serology that utilizes MPV VP2 as antigen is a sensitive and specific assay that differentiates MPV from MVM (Livingston *et al.*, 2002). The MAP test also can be used to detect parvovirus infections but is relatively time-consuming and expensive.

As noted for MVM, PCR for murine parvoviruses, using nucleoprotein gene 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 VP2 gene. Although diagnostic PCR is sensitive and specific, it is effective only in actively infected animals. It can be used on feces to detect virus shedding, or applied to tissues, such as mesenteric lymph nodes, obtained at necropsy.

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

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, but a cage-by-cage test and cull approach has been shown to be successful under natural conditions (Macy *et al.*, 2011). 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. With the increasing use of mouse germplasm, it is important to note that mouse sperm, oocytes, ovarian tissue, and preimplantation embryos from enzootically MPV-infected mouse colonies may have a high prevalence of MPV contamination, based upon PCR (Agca *et al.*, 2007).

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. Murine Adenovirus Infection (Percy and Barthold, 2007; Spindler *et al.*, 2007)

Etiology Adenoviruses are nonenveloped DNA viruses that produce intranuclear inclusions *in vitro* and *in vivo.* Two adenovirus species in the genus *Mastadenovirus* have been associated with mice: *Murine mastadenovirus A* (with the representative strain being MAV-1 or FL) and *Murine mastadenovirus B* (with the representative strain being MAV-2 or K87). Both strains replicate in mouse kidney tissue culture but are antigenically distinct.

Clinical Signs MAV-1 can cause severe clinical disease after experimental inoculation of infant mice. Signs include scruffiness, lethargy, stunted growth, and often death within 10 days. MAV-2 virus is enterotropic and is responsible for virtually all naturally occurring infections in contemporary mouse populations. Infection is usually subclinical in immunocompetent mice, with the possible exception of transient runting among infant mice. Wasting disease can occur in athymic mice infected with MAV-1.

Epizootiology The prevalence of adenovirus infection in mouse colonies is low, particularly MAV-1 (Livingston and Riley, 2003, Pritchett-Corning *et al.*, 2009). Transmission occurs by ingestion. Adult mice experimentally infected with MAV-1 may remain persistently infected and excrete virus in the urine for prolonged periods. Adult mice experimentally infected with MAV-2 excrete virus in feces for at least 3 weeks but eventually recover. Athymic mice can shed MAV-2 for at least 6 weeks and episodically for at least 6 months.

Pathology Infection with MAV-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, with the development of intranuclear inclusions. More mature mice usually develop subclinical infection leading to seroconversion; however, athymic and SCID mice can develop intestinal hemorrhage and wasting, with fatal disseminated infection (Lenaerts *et al.*, 2005). Infection with MAV-2 produces amphophilic, intranuclear inclusions in intestinal epithelium, especially in the distal small intestine (Fig. 3.20). Inclusions are easier to detect in infant mice than in adults. Infection of C.B-17-*Prkdc*^{scid} mice with MAV-2 results in enteric infection, but also hepatic lesions resembling Reye's syndrome (Pirofski *et al.*, 1991).

Diagnosis Although MAV 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. Cross-neutralization tests have revealed that antiserum to MAV-2 neutralizes both strains, but antiserum to MAV-1 neutralizes MAV-2 weakly at best. Therefore, MAV-2 antigen should be used for the serological detection of adenovirus infection irrespective of the assay employed. MAV also can be detected by PCR.

Differential Diagnosis Intranuclear adenoviral inclusions in intestinal epithelium are pathognomonic

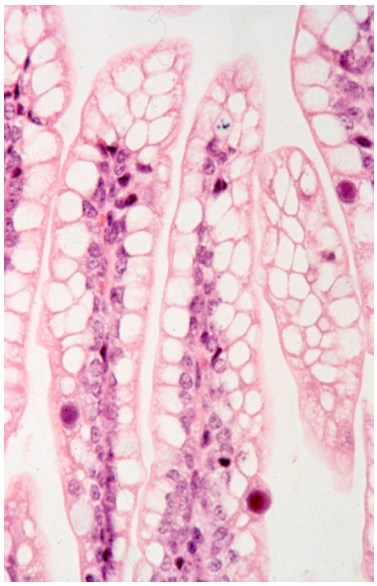

FIGURE 3.20 Intranuclear adenoviral inclusions in the small intestine of an infant mouse naturally infected with mouse adenovirus (MAV B). Enterocytes are also vacuolated, typical of the normal neonatal mouse bowel. *Percy and Barthold (2007).*

and differentiate MAV-2 infection from other known viral infections of mice. Infection may resemble rotavirus infection, with runting and abdominal bloating in infant mice.

Prevention and Control Prevention requires serological monitoring of mice and examination for contamination of animal products such as transplantable tumors. Because MAV-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 MAV infection is unlikely to affect research using immunocompetent mice. However, it has the potential for pathogenicity in immunodeficient mice.

e. Polyomavirus Infections (Benjamin, 2007)

Mice can incur natural infection with two polyomaviruses: polyoma virus (PyV) and K virus. These viruses belong to the family Polyomaviridae. K virus belongs to the genus *Polyomavirus* and the species *Murine pneumotropic virus*, while the classical polyoma virus belongs to the species *Murine polyomavirus*.

POLYOMA VIRUS

Etiology Polyoma virus (PyV) is a small DNA virus that derives its name 'polyoma' (many tumors) from its ability to experimentally induce multiple types of tumors in mice experimentally infected as neonates. Its primary importance stems from use in murine models of experimental oncogenesis, with natural infection being rare. The transformative activity is mediated by 'T' (tumor) antigens, encoded by large T, middle T, and small T genes, with middle T (MT) being considered the major viral oncogene, and as a result has been used extensively in transgenic constructs.

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

Epizootiology Modern husbandry and health care have essentially eliminated natural exposure in laboratory mice. PyV is used for experimental studies and thus can inadvertently be introduced to mouse colonies. Inoculation of mice with contaminated biologicals or cell cultures is a potential source of entry and spread.

Natural transmission occurs via the respiratory route. Exposure of neonatal mice results in persistent infection and shedding of the virus in urine, feces, and saliva, thereby contaminating the environment for spread to other mice. Infection of adult mice is transient, with minimal virus shedding, although PCR has revealed infection lasting up to 5 months in CBA mice inoculated with virus as adults (Berke and Dalianis, 1993). Maternal antibody is highly effective at preventing infection of newborn mice, but as maternal antibody wanes, mice are partially susceptible, with transient virus shedding. Thus, the natural cycle of transmission in enzootically infected populations requires contamination of bedding and nesting material in order to infect and be inefficiently transmitted, which is readily precluded by modern husbandry. Intrauterine infection also can occur, and persistent renal infection, contracted neonatally, can be reactivated during pregnancy. As in immunologically immature neonatal mice, PyV infection can persist in adult immunodeficient mice.

Pathology PyV-induced tumors are essentially a laboratory phenomenon, optimized by virus strain and mouse strain, with AKR, C3H, C58, CBA, SWR, and others being most susceptible, and C57BL/6 being among the most resistant to PyV oncogenesis. Intranasal inoculation of neonatal mice results in initial replication in pulmonary respiratory epithelium (Gottlieb and Villarreal, 2000) followed by viremic dissemination and acute, lethal disease. Tumors appear 2–12 months after inoculation of surviving mice. Tumors of both epithelial and mesenchymal origin arise in multiple organs, particularly mammary carcinomas, basal cell tumors of the skin, carcinomas of salivary glands, thymomas, and various types of sarcomas. Athymic mice can develop cytolytic and inflammatory lesions, followed by multisystemic tumor formation. Intranuclear inclusions may be present in cytolytic lesions. Demyelinating disease and skeletal tumors have been reported in experimentally

inoculated and naturally exposed athymic mice, and myeloproliferative disease has been reported in experimentally inoculated C57BL/6-*scid* mice (Szomolanyi-Tsuda *et al.*, 1994).

Diagnosis PyV can be isolated in mouse fibroblast cell lines, but infection is ordinarily detected serologically. Additionally, PCR and immunohistochemistry can be used.

Differential Diagnosis Wasting in athymic mice can be caused by other infectious agents, including coronaviruses, Sendai virus (SV), and *Pneumocystis.* 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. Biological material destined for mouse inoculation should be tested for PyV by the MAP test or molecular diagnostics.

Research Complications PyV 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.

K Virus Infection K virus has historical importance, and is apparently absent from contemporary mouse populations (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009), but it continues to be tested for, adding to the expense of infectious disease surveillance. 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 the lung, liver, spleen, and adrenal glands. Dyspnea occurs from pulmonary infection because of edema and hemorrhage. Infection of immunocompetent adult mice is subclinical and results in a vigorous immune response. However, both adults and infant mice develop persistent infection. The primary organ for persistence is the kidney, with shedding of virus from tubular epithelium, and shedding can be reactivated by immunosuppression (Greenlee *et al.*, 1991). 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 hepatocyte degeneration also may develop. Infection can be detected by serology or PCR. Prevention and control measures, if ever to be found within a mouse population, are similar to those described for PyV.

f. Lactate Dehydrogenase–Elevating Virus Infection (Coutelier and Brinton, 2007)

Etiology Lactate dehydrogenase–elevating virus (LDV) is a mouse-specific small enveloped RNA virus belonging to the family Arteriviridae. Infected mice are persistently viremic, resulting in increased concentration of several serum enzymes, most notably lactate dehydrogenase (LDH). Infection is common among wild mice, but is now rare in contemporary laboratory mouse populations. However, surveys of biologic material indicate that LDV may be a common contaminant of biologic materials (Nicklas *et al.*, 1993).

Clinical Signs Infection is subclinical. However, poliomyelitis has occurred in immunosuppressed C58 and AKR mice inoculated with LDV, and has recently been observed in ICR-*scid* mice following inoculation with contaminated biologic material (Carlson-Scholz *et al.*, 2011).

Epizootiology 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. LDV selectively targets mature F4/F8-positive macrophages, which are continually produced by uninfected progenitor cell populations, thereby maintaining persistent infection. Virus also escapes immune clearance by evolution of neutralizing antibody-resistant quasi-species. No lesions are seen in naturally infected mice. The only significant lesion that can arise from experimental infection is poliomyelitis. This syndrome requires a combination of immunosuppression (due to age, genetics or induced means), mouse strain (C58, AKR, C3H/Fg, and PL), neurotropic LDV strains, and endogenous ecotropic murine leukemia virus. The mouse strain-dependent element is homozygosity for the *Fv-1*^{n} allele, which permits replication of endogenous N-tropic ectotropic murine leukemia virus. Mice develop spongiosis, neuronal necrosis, and astrocytosis of the ventral spinal cord and brain stem, with axonal degeneration of ventral roots. Lesions contain both LDV and retrovirus. Although this syndrome is largely experimentally induced, a natural outbreak of poliomyelitis has been reported in *Fv-1* homozygous ICR-*scid* mice following inoculation with contaminated biologic material (Carlson-Scholz *et al.*, 2011).

Diagnosis Plasma LDH levels are elevated, a response that is used to detect and titrate LDV infectivity. Of the five isoenzymes of LDH in mouse plasma, only

LDH-V 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 nonhemolyzed 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. In a recent survey, 6000 serum samples were tested by serum LDH enzyme assays, among which 10% were deemed potentially positive. However, PCR revealed that all were false-positives (Pritchett-Corning *et al.*, 2009), emphasizing the inaccuracy of traditional enzyme assays.

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 biologic materials. 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 contaminate tumor cell lines, it does not replicate in the tumor cells. Therefore, one can attempt to free tumors of virus by passaging them through athymic nude rats, which are not nonpermissive to LDV but are permissive to xenografts.

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 Infection (Barthold and Smith, 2007)

Etiology The house mouse is the natural host for lymphocytic choriomeningitis virus (LCMV), an Old World member of the Arenaviridae family that has spread worldwide along with *M. musculus*. LCMV virions are pleomorphic, containing single-stranded RNA, and bud from the cell membrane. Disease associated with infection is due to host immune response to the otherwise non-cytolytic virus. Its name is derived from the immune-mediated inflammation resulting from the intracerebral inoculation of virus into immunologically competent mice. LCMV is a zoonotic virus that may cause a variety of clinical manifestations in humans, including meningitis. It has been extensively studied as an experimental model of virus-induced immune injury, using a number of closely related strains, including ones that have been selected for their relative neurotropism or viscerotropism. LCMV can be propagated in a variety of mammalian, avian, and even tick cell lines, with minimal cytopathic effect. These characteristics favor its propensity to persistently and silently contaminate biologic products, such as tumor cell lines.

Clinical Signs Natural infection in immunocompetent adult mice is usually self-limiting and subclinical. During enzootic infection of a mouse population, LCMV is transmitted *in utero* from persistently infected dams to their fetuses or to neonates, which are persistently infected and immunologically tolerant to LCMV. Since LCMV is non-cytolytic in and of itself is minimally pathogenic, congenitally infected mice grow into adulthood, reproduce, and therefore transmit infection to the next generation. However, with age, immune tolerance breaks down, and mice develop a syndrome known as 'late disease' in which mice will progressively lose weight and die. *In utero* infection results in a low level of fetal mortality and maternal cannibalism of infected pups. The immune tolerance to LCMV is virus-specific, with the mice capable of eliciting effective immune responses against other agents.

Clinical signs following experimental inoculation of LCMV vary with age and strain of mouse, route of inoculation, and strain of virus. When virus is inoculated intracerebrally into immunocompetent adult mice, mice develop immune-mediated lymphocytic choriomeningitis, characterized by illness beginning 5–6 days after inoculation. 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 rear legs. Spontaneous convulsions also can occur. Animals usually die or recover in several days. A visceral form of infection can occur in adult mice inoculated by peripheral routes with 'viscerotropic' strains. It can be subclinical or lead to clinical signs, including ruffled fur, conjunctivitis, ascites, somnolescence, and death. If mice survive, recovery may take several weeks. Surviving mice may have immune exhaustion due to consumption of lymphoid tissue, in contrast to immune tolerance that occurs when mice are infected *in utero* or as neonates. Runting and death from LCMV infection may occur in neonatally infected 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. Late-onset disease can occur in previously subclinical 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 *M. musculus* throughout the world. 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 rare in laboratory mice produced and maintained in modern quarters (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). 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. Naturally infected carrier mice can have persistently high concentrations 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, in 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. 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, noncytolytic endocrine dysfunction, and immune complex disease. However, lesions comparable to experimentally induced disease are rare during natural infection. Intracerebral inoculation of virus into immunocompetent adult mice induces 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 hepatocyte necrosis accompanied by nodular infiltrates of lymphoid cells and Kupffer 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 immunopathologic processes. In adult mice with acute LCMV infection, virus multiplies in DCs, 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. 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. Tissues, including biologic products and cell lines, can be tested

by PCR. A traditional method for detection involved collection of small blood samples from persistently infected live suspects, which are often viremic, and using them 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 subclinical. 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 MAP test. 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 (see Chapter 29). 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 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. Infection of immunodeficient mice poses similar risk. LCMV can be transmitted to human beings, who can contract flu-like illness or severe CNS disease. More frequently, human infection is subclinical. The zoonotic potential of LCMV infection makes it especially important 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, are 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 risk to humans are obvious research-related hazards.

h. SV Infection (Brownstein, 2007)

Etiology 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. Once common in laboratory rodent populations, SV is now rare or absent (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009).

Clinical Signs Clinically affected 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 subclinical infection. Athymic mice and immunodeficient mice are at high risk for development of a wasting syndrome. They develop illness later than their immunocompetent counterparts, since clinical signs in immunocompetent mice are related to immune-mediated destruction of respiratory epithelium. Opportunistic infections can complicate the clinical presentation. For example, secondary bacterial infections of the ear can cause vestibular signs.

Epizootiology SV is transmitted by aerosol and is highly contagious. 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 immunocompetent 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 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. 3.21). 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 pneumocytes are less severely affected. Histologically, the pattern of pneumonia is influenced by mouse genotype. Susceptible mice usually have significant bronchopneumonia and interstitial pneumonia, whereas the interstitial component may be less prominent in resistant mice. Typical changes begin with inflammatory edema of bronchiolar lamina propria, which may extend to alveolar ducts, alveoli, and perivascular spaces. Necrosis and exfoliation of bronchiolar epithelium ensue, frequently in a segmental pattern (Fig. 3.22). 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 (Fig. 3.23). Lymphoid cells also invade peribronchiolar and perivascular spaces. The lymphocytic response to SV infection reflects the fact that cellular immunity contributes both to lesions and to recovery. 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 clinically susceptible hosts mounting a more florid immune 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 SCID mice, 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

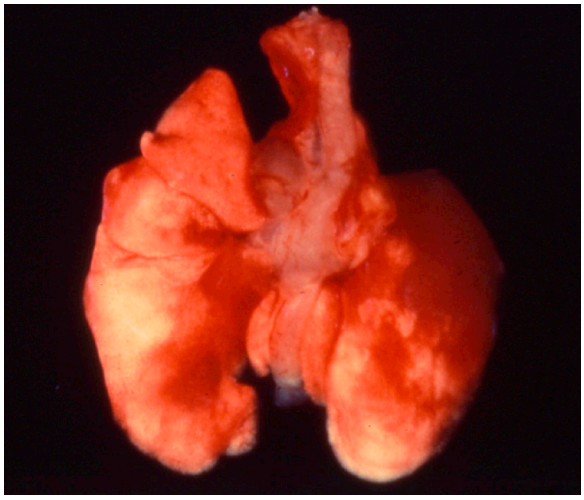

FIGURE 3.21 Lungs from a DBA mouse infected with SV. Note consolidation of lung tissue at the hilus. *Percy and Barthold (2007).*

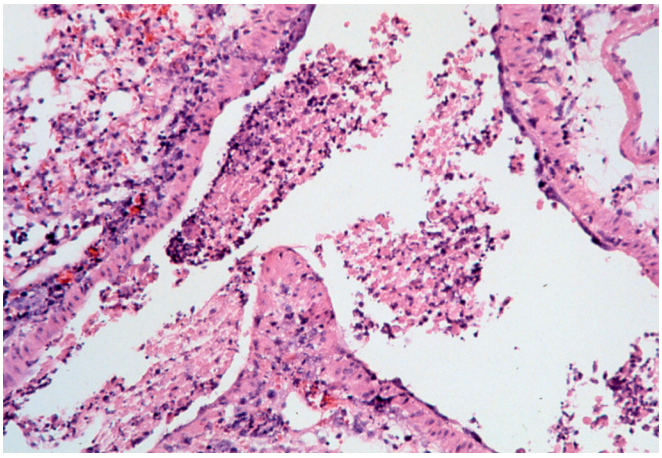

FIGURE 3.22 Necrotizing bronchiolitis in a DBA mouse infected with SV. *Courtesy of S.W. Barthold.*

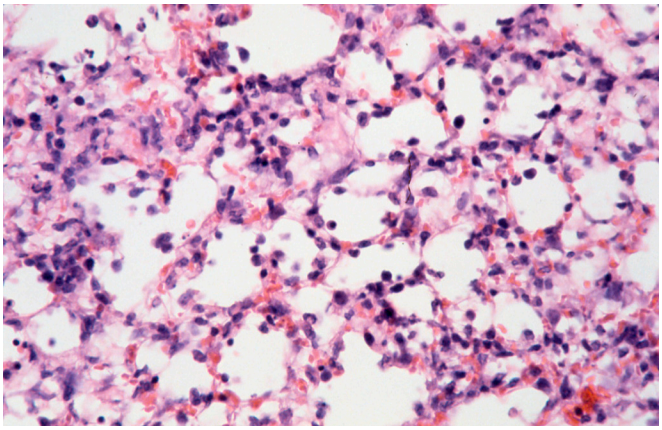

FIGURE 3.23 Interstitial pneumonia in a DBA mouse infected with SV. *Courtesy of S.W. Barthold.*

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 SV is notable for its ability to cause epizootics of acute respiratory distress in adult genetically susceptible strains. Serology is an effective means to detect infection in all strains of immunocompetent mice. Antibody can be detected by 7 days postinfection and coincides with development of clinical signs related to the immune-mediated necrotizing bronchiolitis and alveolitis. Repeated serologic sampling over several weeks can help stage infection within a population. 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 non-cytolytic, so erythrocyte agglutination or antigen detection methods must be used. RT-PCR also can be used to detect virus in infected lungs.

Differential Diagnosis Respiratory infection caused by pneumonia virus of mice (PVM) is generally milder or subclinical. 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 SV 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 SV 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 SV 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. Murine Pneumonia Virus Infection (Brownstein, 2007; Dyer *et al.*, 2012)

Etiology Murine pneumonia virus (PVM) is an enveloped RNA virus in the genus *Pneumovirus* and species *Murine pneumonia virus* of the Paramyxoviridae family. All isolates appear to have similar physicochemical, biological, and antigenic properties, but virulent strains have been selectively developed for experimental use. The virus agglutinates erythrocytes of several rodent species, including mice. It replicates well *in vitro* in BHK-21 cells but, as with SV, is non-cytolytic in cultured cells.

Clinical Signs Natural PVM infection in mice is subclinical. 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). PVM is used experimentally as a model to study acute respiratory infection, using highly pathogenic strains of the virus (Dyer *et al.*, 2012).

Epizootiology PVM causes natural infections of mice, rats, hamsters, and probably other rodents and may be infectious for rabbits. Serological data indicate that PVM was once common, but is now relatively uncommon (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). PVM appears to spread less rapidly than SV. Intimate contact between mice is probably required for effective transmission. This characteristic may reflect the fact that environmental inactivation of virus occurs rapidly. Infection is 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 occur within 2 weeks after exposure to virus and are largely resolved by 3 weeks. The predominant inflammatory infiltrate is comprised of mononuclear cells, but some neutrophils

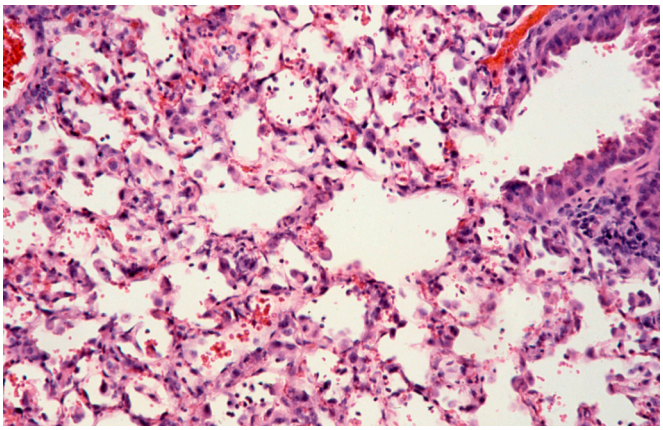

FIGURE 3.24 Severe interstitial pneumonia in an athymic mouse chronically infected with pneumonia virus of mice (MPnV). *Courtesy of S.W. Barthold.*

are usually present. Immunohistochemistry on paraffin-embedded tissues can be used to detect viral antigen in bronchiolar epithelium, alveolar macrophages, and alveolar epithelium during acute infection. Residual lesions include nonsuppurative perivasculitis, which can persist for several weeks after acute infection has ceased. Severe progressive pneumonia, with wasting, can occur in immunodeficient mice. It is characterized by generalized pulmonary consolidation that reflects severe interstitial pneumonia with desquamated alveolar pneumocytes and leukocytes filling alveolar spaces (Fig. 3.24).

Diagnosis Diagnosis is based primarily on serological detection that can be supplemented by histopathology, immunohistochemistry, *in situ* hybridization, and virus isolation. 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.

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 and lesions are typical, it must be differentiated from other pneumonias, especially those due to SV and *Pneumocystis.* Additionally, PVM can coexist with and exacerbate *Pneumocystis* infection in immunodeficient mice (Bray *et al.*, 1993).

Prevention and Control PVM infection is acute and self-limiting in immunocompetent mice, but persistent in immunodeficient 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 (Ward *et al.*, 2007)

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

MAMMALIAN ORTHOREOVIRUS (REOVIRUS 1, 2, AND 3)

Etiology Reoviruses of mammals, although taxonomically considered one type species, have been divided into three cross-reacting prototypic serotypes: reovirus 1, 2 and 3, which can be differentiated by cross-serum neutralization. Mice can be infected with any serotype, but reovirus 3 is emphasized because it has been associated with naturally occurring disease. Natural infections in mice are usually not caused by pure serotypes, because reoviruses actively recombine. A number of wild-type and laboratory strains have been characterized, and related viruses have been recovered from virtually every mammal tested, as well as birds, reptiles, and insects. The virion contains segmented, double-stranded RNA and is relatively heat stable. 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 have subclinical 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 contemporary mouse colonies is rare (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). Reoviruses are highly contagious 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. 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 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 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 have a vascular distribution, and 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 hepatocyte necrosis, which may include the accumulation of dense eosinophilic structures resembling Councilman bodies. Hepatocytomegaly, Kupffer 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 the 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.

Oronasal inoculation of infant mice with reovirus 1 results in a similar distribution but significantly milder lesions compared to reovirus 3. In contrast, reovirus 2 is highly enterotropic, inducing mild enteritis without lesions in other tissues, similar to Epizootic Diarrhea of Infant Mice (EDIM) (Barthold *et al.*, 1993).

Diagnosis Serology uses reovirus 3 as antigen, which detects seroconversion to all serotypes, and viral RNA can be detected by RT-PCR. A presumptive diagnosis of reovirus 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 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 transplantable neoplasms and is suspected of being oncolytic. The potential exists for interference with hepatic, pancreatic, cardiovascular, or neurological research.

ROTAVIRUS (Barthold, 1997a; Ward et al.*, 2007)*

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. The cardinal signs are bloated abdomens 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, and infection is probably widespread geographically (Livingston and Riley, 2003; Pritchett-Corning

et al., 2009). All ages and both sexes can be infected, but genetic resistance and susceptibility have not been determined. The virus is highly contagious and is transmitted by the oral–fecal route. Subclinically infected adult mice can shed virus in feces for at least 17 days, an interval that may be extended in immunodeficient mice. After oral inoculation, virus is essentially restricted to the gastrointestinal tract, although small amounts of virus may be present in the 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 involution 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 due to caking of dried feces. Virus preferentially infects terminally differentiated enterocytes in the small and large intestines, which accounts for the age-related susceptibility to disease; the number of such cells decreases as the intestinal tract matures. Characteristic histological lesions are often very subtle, but are most easily discerned in the small intestine in mice less than 2 weeks old. They consist of vacuolation of villar epithelial cells with cytoplasmic swelling, which give villi a clubbed appearance (Fig. 3.25). 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 readily detected serologically. 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 the intestine or in intestinal filtrates or smears. Rotavirus antigen can be detected in feces by ELISA, but certain dietary ingredients can cause false-positive reactions. Infection can also be diagnosed by RT-PCR.

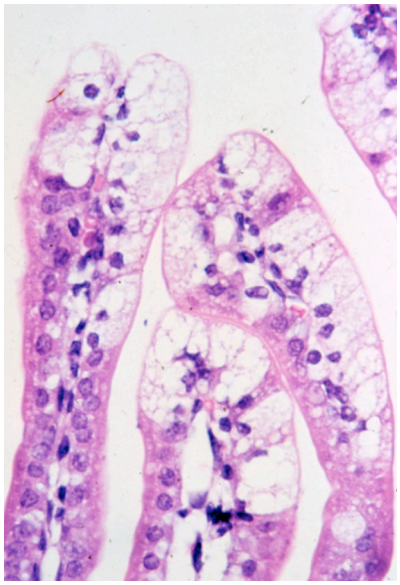

FIGURE 3.25 Mouse rotavirus (EDIM) infection. Note swelling of enterocytes at the tips of villi. *Percy and Barthold (2007).*

Differential Diagnosis EDIM virus infection must be differentiated from other diarrheal diseases of suckling mice such as intestinal coronavirus (mouse hepatitis virus) 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 or other stressors.

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. If EDIM virus is allowed to become enzootic within a colony, clinical signs will disappear within the population, which may be an appropriate management approach in conventional colonies. 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. Murine Coronavirus (Mouse Hepatitis Virus) Infection (Barthold, 1997a,b)

Etiology Coronaviruses are large, pleomorphic, enveloped RNA viruses with radially arranged peplomers (spikes). In mice, 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). During that time, enteritis in infant mice was recognized as a separate entity caused by an uncharacterized virus, known as Lethal Intestinal Virus of Infant Mice (LIVIM). Subsequent studies revealed that hepatitis-causing MHV and

enteritis-causing LIVIM were closely related coronaviruses, now collectively termed MHV. MHV isolates differ in biologic behavior according to their organ tropism into two biotypes: *enterotropic* strains, which infect primarily the intestinal tract, and *polytropic* strains, which initially infect the respiratory tract but may progress to multisystemic dissemination, including the liver and brain. These differences are often reflected in their cell tropism *in vitro*. However, natural isolates may contain features of both biotypes.

Several prototype polytropic strains have been extensively studied as experimental models of hepatitis and encephalitis. They include JHM (MHV4), MHV-1, MHV-3, MHV-S, and MHV-A59. Numerous additional strains have been identified that differ in virulence, tissue tropism, and antigenicity. Differentiation by strain, particularly under natural conditions, is irrelevant, since mutation is common among coronaviruses, and even named prototype strains differ significantly depending upon passage history. 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 may be used for propagating polytropic 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. MHV can also be grown in mouse macrophages, cells that have been used for genetic studies of resistance and susceptibility to infection. Enterotropic strains, because of their tendency to be strictly enterotropic, 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. Irrespective of cellular substrate used for isolation or propagation, syncytium formation is emblematic of MHV infection (Fig. 3.26).

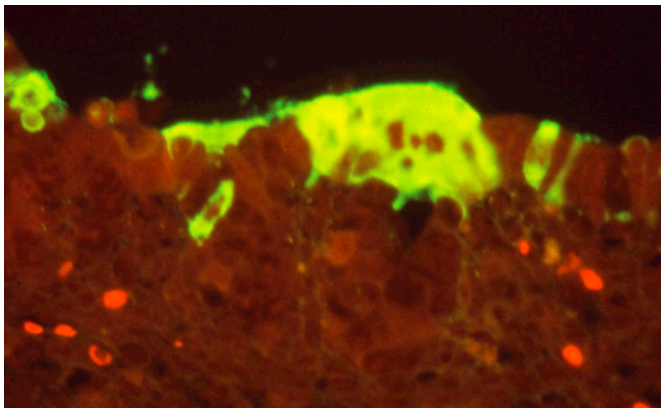

FIGURE 3.26 Immunofluorescent staining of MHV-infected intestinal mucosa. Note the syncytium in the center, typical of MHV. *Courtesy of S.W. Barthold.*

FIGURE 3.27 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).

Clinical Signs Clinical signs depend primarily on the age, strain, and immunological status of infected mouse and strain and tropism of virus. 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 infected with enterotropic MHV develop inappetence, diarrhea, and dehydration, often terminating in death (Fig. 3.27). Epizootics of enterotropic MHV have been known to result in 100% mortality among neonatal mice in a breeding colony. Older mice (2–3 weeks of age) may have ruffled pelage and runting. Neurotropic strains such as MHV-JHM may induce flaccid paralysis of the hindlimb, but this sign is rarely encountered alone during natural infection. Conjunctivitis, convulsions, and circling may be seen occasionally. Enterotropic strains may not cause acute disease in athymic mice when exposed as adults, whereas mildly pathogenic polytropic strains can cause a progressive wasting syndrome that may be accompanied by progressive paralysis.

Epizootiology MHV infection, despite constant surveillance and preventive programs, continues to be a common threat to laboratory mouse populations (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). There are no reports of natural transmission from mice to other species, but suckling rats have been found to develop necrotizing rhinitis after intranasal inoculation with MHV-S. MHV is highly contagious, with natural transmission occurring by respiratory or oral routes.

Enterotropic biotypes predominate in natural infections in contemporary laboratory animal facilities, since they tend to be the most contagious due to copious excretion of virus in feces, whereas polytropic strains generally spread by direct respiratory contact. 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 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, but intestinal proliferative kinetics are critical determinants of disease susceptibility with enterotropic MHV. 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 pressure 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. 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 susceptibility of young mice to infection increases significantly at weaning.

Strain differences in resistance and susceptibility to polytropic MHV can be inherited as an autosomal dominant trait. 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 is also 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 (IFN-γ) 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. 3.28). 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 the intestine (Fig. 3.26), 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. 3.29). 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 progressive 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

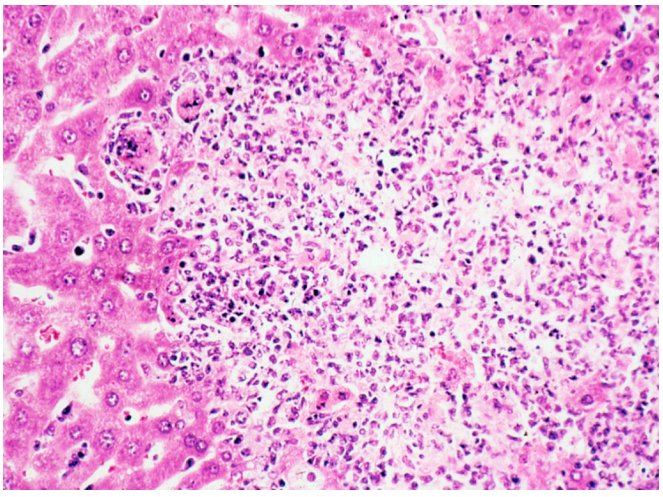

FIGURE 3.28 Necrosis, inflammation, and syncytium in the liver of a mouse infected with MHV. *Courtesy of S.W. Barthold.*

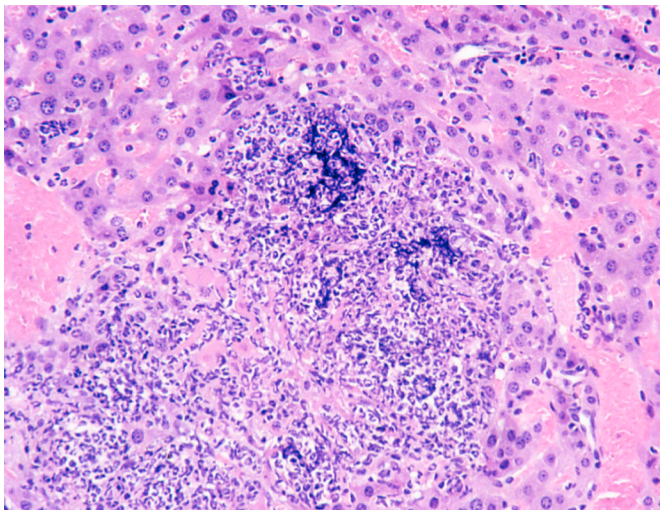

FIGURE 3.29 Hepatitis and syncytia in the liver of an SCID mouse. Note the much more obvious syncytia in this liver due to absence of immune response, compared to liver of an infected immunocompetent mouse in Figure 3.28. *Courtesy of S.W. Barthold.*

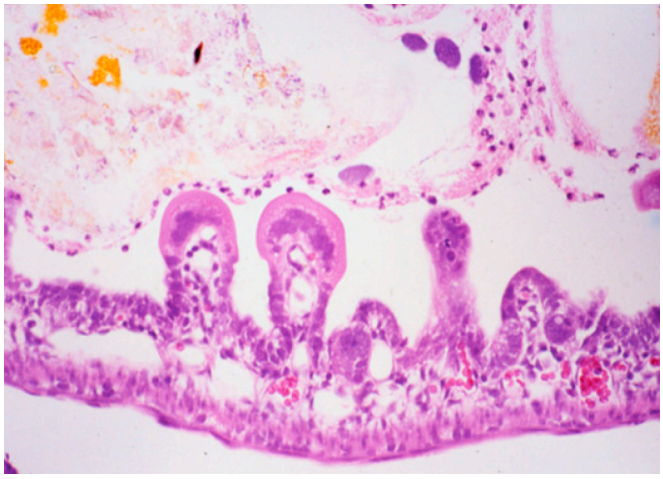

FIGURE 3.30 Small intestine of neonatal mouse infected with enterotropic MHV. Villi are markedly attenuated and there are prominent syncytia at the tips of villi. Percy and Barthold (2007).

cause systemic lesions, especially in the liver and brain. The most common sites are terminal ileum, cecum, and proximal colon. The severity of disease is age-related, and dependent upon intestinal proliferative kinetics, similar to EDIM, with young infants being at highest risk for lethal infection. Pathogenic strains can cause lesions ranging from villus attenuation to fulminant necrotizing enterotyphlocolitis, which can kill suckling mice within a few days (Fig. 3.27). The stomach is often empty, and the intestine is filled with watery to mucoid yellowish, sometimes gaseous contents. Syncytia are a

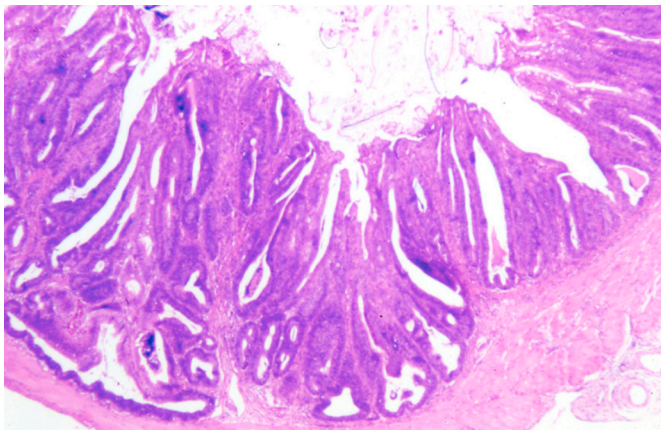

FIGURE 3.31 Proliferative colitis in an athymic mouse chronically infected with enterotropic MHV. *Courtesy of S.W. Barthold.*

consistent feature in viable mucosa (Fig. 3.30) and not only are formed in intestine but also may be present 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, but may contribute to clinical signs due to osmotic, secretory, and malabsorptive diarrhea. Older mice are equally susceptible to infection, but are resistant to severe disease due to their mature (more rapid) intestinal proliferative kinetics. Pathology may be subtle, consisting of transient syncytia without necrotic lesions. In adult mice, syncytia can be found most often in the surface mucosal epithelium of the ascending colon. The exception occurs in immunodeficient mice, such as athymic and SCID mice, which can develop chronic proliferative bowel disease of varying severity with MHV antigen in mucosal epithelium (Figs. 3.31 and 3.32). This may not always be present, as athymic nude mice exposed as adults may only manifest a few enterocytic syncytia without hyperplasia.

Diagnosis Because MHV infection is often subclinical, serological testing is the most reliable diagnostic tool. Many animal resources rely on sentinel mouse protocols for continuous serological surveillance. Serology is well established, sensitive, and reliable. Neutralization tests are used to differentiate individual virus strains in the research laboratory but are inappropriate for routine use, because of cost, technical complexity, and serologic identification *per se* does not predict biological behavior, including virulence or tissue tropism. Serology also can be used in the context of MAP testing in which adult mice are inoculated with suspect tissues to elicit seroconversion. RT-PCR protocols to detect virus in tissues or excreta are available. The detection of syncytia augmented, when possible, by immunohistochemistry to

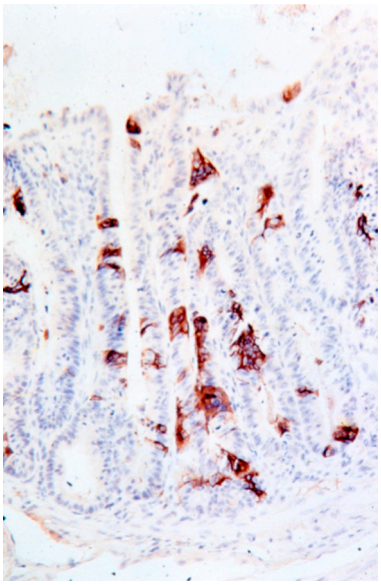

FIGURE 3.32 MHV antigen in colonic mucosa of an athymic mouse chronically infected with enterotropic MHV. *Courtesy of S.W. Barthold.*

detect MHV antigen is a useful and practical means to confirm infection. 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, 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 virus-induced tumors in athymic mice.

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, selective quarantine at the cage (not room) level with the temporary cessation of breeding can be used effectively to eliminate infection. Quarantine at the room basis is likely to fail, since mutations arise and continually reinfect the mouse population. Additionally, maternally derived immunity can protect infant mice from infection until they are weaned and moved to uncontaminated quarters. Careful testing with sentinel mice should be used to assess the effectiveness of quarantine or 'natural rederivation,' as just described. Immunodeficient mice, in contrast, are susceptible to chronic infection and viral excretion. Mice with unrecognized or unanticipated immune dysfunction or with selective immune dysfunction may impact on MHV infection and its control. Such colonies, which may contain highly valuable or irreplaceable 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 maintenance 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, and the unpredictable outcome of infection in genetically altered mice is likely to lengthen the list. For example, apart from its clinical impact, MHV may stimulate or suppress immune responses, contaminate transplantable neoplasms, and be reactivated by treatment of subclinically 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 subclinical or strictly enterotropic infection should be taken lightly but simply to caution against overreaction in assessing the impact of an outbreak.

l. Theiler's Murine Encephalomyelitis Virus Infection (Lipton *et al.*, 2007)

Etiology Mice are susceptible to infection by two members of the *Cardiovirus* genus within the

Picornaviridae family, including a virus in the species *Encephalomyocarditis virus* (EMCV) and Theiler's murine encephalomyelitis virus (TMEV), a virus in the species *Theilovirus*. EMCV has a less selective host range and can infect wild mice, but is not known to infect laboratory mice. TMEV is a small, nonenveloped, RNA virus that was discovered by Max Theiler during experimental studies of yellow fever virus in mice. Established prototype 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. TMEV 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. TMEV is antigenically related to EMCV. As with other nonenveloped viruses, TMEV 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 natural disease 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 that are otherwise alert (Fig. 3.33). 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 TMEV in mouse colonies is low, a reflection of the slow rate at which virus is transmitted from mouse to mouse, but it continues to be among the more common viral contaminants of mouse colonies (Livingston and Riley, 2003; Pritchett-Corning *et al.*, 2009). TMEV 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. Immunity to one strain of TMEV 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.

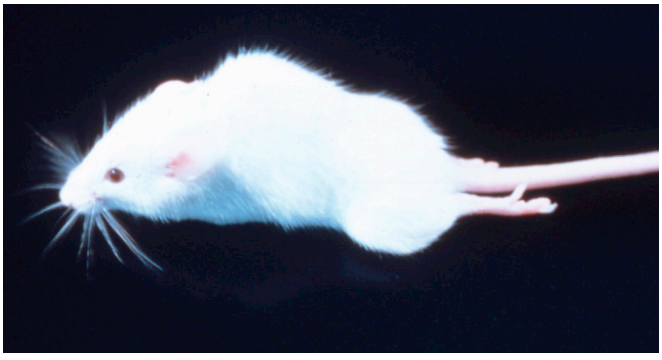

FIGURE 3.33 Posterior paralysis in a mouse naturally infected with mouse encephalomyelitis virus (MEV).

Pathology Intestinal TMEV 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 and neurons, neuronophagia, and perivascular inflammation, which occur 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. 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 or by PCR of feces, but virus shedding from infected mice may be intermittent. 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.

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. Polyoma virus infection in athymic mice can induce tumors or demyelination in the CNS, which may result in clinical signs resembling those of TMEV infection.

Prevention and Control Disease-free stocks were originally developed by foster-nursing infant mice. This technique, cesarean rederivation, or embryo transfer can be used successfully to eliminate infection. 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 TMEV for research relates to its potential effects on the CNS.

m. Mouse Norovirus Infection (Wobus *et al.*, 2006)

Noroviruses are nonenveloped RNA viruses that belong to the family Caliciviridae. They are notoriously resistant to environmental inactivation, and cause significant gastrointestinal morbidity in humans. Noroviruses are species-specific, including MNV, which exclusively infects mice. Until the discovery of MNV, replication of noroviruses *in vitro* has not been possible. For this reason, MNV has emerged as an important small animal model of norovirus pathogenesis. MNV was relatively recently discovered in 2003, and subsequent surveillance has revealed that it is the most common adventitious virus infection in laboratory mice (Hsu *et al.*, 2005; Pritchett-Corning *et al.*, 2009). Over 35 MNV isolates have been found in mouse research colonies around the world, which display nearly 90% genetic identity, comprising a single genetic cluster. Although genetically homogeneous, significant biological differences exist among MNV strains (Thackray *et al.*, 2007). MNV effectively replicates in macrophages and dendritic cells, including the mouse macrophage-like RAW264.7 cell line, as well as a microglial cell line (Wobus *et al.*, 2004).

Clinical Signs Clinical signs of infection in immunocompetent mice are usually absent, but infection leads to systemic disease with high mortality in interferon αβγ receptor and STAT1 null mice. Affected mice have loss of body weight, ruffled fur, and hunched posture (Ward *et al.*, 2006). Experimental infection of 129 and C3H mice with MNV-1 caused mild diarrhea (Kahan *et al.*, 2011).

Epizootiology MNV is transmitted by the fecal–oral route, and contaminates the environment as an environmentally resistant virus. For this reason, it can efficiently infect sentinel mice with soiled bedding (Manuel *et al.*, 2008). Duration of infection varies with MNV strain, mouse immunocompetence and mouse genotype. Experimental studies have revealed that several MNV strains persist in various tissues of C57BL/6J, Hsd:ICR, and Jcl:ICR and C.B-17-*Prkdc*^{scid} mice, with fecal shedding for at least 35–60 days (Goto *et al.*, 2009; Hsu *et al.*, 2006; Thackray *et al.*, 2007). Although not clinically ill, RAG1 null mice are unable to clear infection (Wobus *et al.*, 2006). Comparative studies with MNV-1 and MNV-3 have shown differences in virus replication and shedding (Kahan *et al.*, 2011). MNV has a tropism for macrophages and dendritic cells, and virus can be detected in the intestine, intestinal lymphoid tissue, liver, and spleen (Hsu *et al.*, 2006; Kahan *et al.*, 2011; Wobus *et al.*, 2006).

Pathology Naturally and experimentally infected STAT1 or IFNγR null mice may develop splenomegaly and multifocal pale spots on the liver. Microscopic findings include varying degrees of hepatitis, focal interstitial pneumonia, vasculitis, peritonitis, and pleuritis (Karst *et al.*, 2003; Ward *et al.*, 2006). Encephalitis, cerebral vasculitis, pneumonia, and hepatitis have also been described in intracerebrally infected STAT1 null mice (Karst *et al.*, 2003). Infection of immunocompetent mice may be associated with mild inflammation of the intestine, splenic hypertrophy, and lymphoid hyperplasia of spleen and lymph nodes (Mumphrey *et al.*, 2007).

Diagnosis MNV infection can be detected by serology or RT-PCR. Sentinel mouse surveillance, using soiled bedding, is an effective strategy for detecting MNV (Manuel *et al.*, 2008)

Differential Diagnosis The mild change in fecal consistency associated with MNV in adult mice may mimic rotavirus, coronavirus, *Helicobacter* spp., *Citrobacter rodentium*, or other enteric diseases. Disseminated lesions in STAT1 or IFNγR null mice must be differentiated from other polytropic viral diseases in immunodeficient mice, including MHV.

Prevention and Control Depopulation and decontamination has been shown to be effective at eliminating MNV from an enzootically infected colony, whereas test-and-removal of positive mice was found to be ineffective (Kastenmayer *et al.*, 2008). Embryo transfer and cesarean rederivation are also effective (Goto *et al.*, 2009; Perdue *et al.*, 2007). Neonatal mice are resistant to infection, so that cross-fostering neonates onto uninfected dams is another effective means of rederivation MNV-free mice (Artwohl *et al.*, 2008; Compton, 2008).

Research Complications The tropism of MNV for macrophages and dendritic cells is likely to modify immune responses, and MNV infection may interfere with studies involving enteric disease.

n. Hantavirus Infection (MacLachlan and Dubovi, 2011)

Hantaviruses are RNA viruses belonging to the very large Bunyaviridae family. They differ from other members of this family by not being arthropod-borne. Each hantavirus is antigenically distinct and maintained within single or at most a few rodent or insectivore hosts, but are infectious for other hosts. Infection is lifelong, and virus is transmitted by shedding of virus in urine, feces, and saliva. Several hantaviruses are zoonotic and may cause severe disease in humans. Although there is overlap, hantaviruses in Asia and Europe cause *hemorrhagic fever with renal syndrome* (HFRS) in humans, a multisystem disease with significant renal involvement, and hantaviruses that are endemic in the Americas cause

hantavirus pulmonary syndrome (HPS) in humans, which is a multisystem disease with pulmonary involvement. Among the better-known Old World HFRS hantaviruses are Hantaan, Seoul, Puumala, and Dobrava–Belgrade viruses. Sin Nombre virus is the best known New World PHS hantavirus, among many others. Most notably from the perspective of laboratory animal medicine, the Norway rat, *Rattus norvegicus*, serves as a reservoir host for hantavirus in the wild, but infection has also been associated with laboratory rats. In addition to being endemic in wild rats in Asia, it has been found to be endemic in wild rats in the eastern United States and associated with human cases of HFRS (Childs *et al.*, 1988; LeDuc *et al.*, 1984; Tsai *et al.*, 1985). Over 120 cases of hantavirus infection have been transmitted to humans from laboratory rats in Japan, Belgium, and the United Kingdom (Desmyter *et al.*, 1983; Kawamata *et al.*, 1987; Lloyd *et al.*, 1984; Umenai *et al.*, 1979). *M. musculus* is not considered to be a primary reservoir host, but hantavirus infection has been documented serologically in conventional and barrier-maintained laboratory mice and rats in Korea (Won *et al.*, 2006), infection of wild *M. musculus* has been documented in the United States (Baek *et al.*, 1988), and infection of wild mice in Europe has been associated with human exposure (Diglisic *et al.*, 1994). Hantaviruses are difficult to culture *in vitro*. Infection in rodents is subclinical and is detected by serology or RT-PCR. The main research complication from natural infection is the zoonotic risk and potentially subclinical effects on the immune response associated with viral defenses such as $CD8^+$ T cell (Taruishi *et al.*, 2007) and NK function as demonstrated in human studies (Braun *et al.*, 2014).

o. Retrovirus Infection (Mammary Tumor Viruses and Mouse Leukemia Viruses) (Coffin *et al.*, 1997; MacLachlan and Dubovi, 2011; Morse, 2007b)

The mouse is host to a number of enveloped RNA viruses of the family Retroviridae, subfamily Orthovirinae, including the two type species (and their variants) *mouse mammary tumor virus* (MMTV) and *murine leukemia virus* (MLV). These viruses belong to a diverse assemblage of related mobile DNA elements that are integrated into the host genome, and collectively termed 'retroelements', which include retrovirus-related elements and nonviral elements. During cell division, retroelements are transcribed into RNA, and subsequently reverse-transcribed into DNA copies that become integrated into a new location within the genome. This process utilizes reverse transcriptase, which is encoded by the retroelement. Over millennia, retroelements have been repeatedly integrated within the genome in large numbers, comprising approximately 40% of the mammalian genome. Various families of mouse retroelements share sequence similarity, despite their random distribution throughout the mouse genome, and the majority of them are truncated, mutated, and methylated to become incapable of infectivity. Nevertheless, many of them continue to be mobile within the genome. Noninfectious retrovirus-related retroelements include IAP, VL30, MusD, and ETn elements.

Replication-competent retroviruses represent the pinnacle of the retroelement constellation and are best considered as the most evolutionarily recent members. These include MMTV and MLV. MTV and MLV share similar genetic structure, except that the long terminal repeat (LTR) region of the MMTV genome encodes an additional superantigen (*Sag*). Both MMTV and MLV include *exogenous* viruses, which are horizontally transmitted, replication-competent viruses, and *endogenous* viruses, which are closely related to exogenous viruses, encoded within the mouse genome, and transmitted by Mendelian inheritance. Exogenous MMTV and MLV exist in wild mouse populations, but have been eliminated from contemporary laboratory mice. However, they may continue to be used experimentally, including Bittner MMTV, and Gross, Friend, Moloney, and Rauscher MLVs. In particular, mouse colonies may be purposely infected with MMTV for mammary cancer research and are termed 'MMTV-positive', reflecting their exogenous virus status, even though the mice may also carry endogenous MMTV.

The genomes of all inbred strains of mice encode one or more (over 50 in some mouse strains) endogenous MMTV loci, the distribution of which is unique to each inbred strain of mouse. Most MMTV genomic loci do not encode infectious virus or are transcriptionally inactive, except for mouse strains (DBA, C3H, GRS) that carry *Mtv1 or Mtv2* loci. These loci encode infectious virus, which can be visualized as B-type particles by electron microscopy. Likewise, all mouse strains carry endogenous MLV loci within their genome, but not all mice carry replication-competent MLV sequences. Some endogenous MLVs encode infectious virus, which can be visualized as C-type particles by electron microscopy. Mice have often evolved mechanisms to counter the deleterious effects of retroviruses by preventing reentry or replication of virus into other cells. If an endogenous retrovirus is still infectious to other mouse cell targets, it is termed *ecotropic*, whereas if it is no longer infectious for mouse cells, but can infect cells of other species, it is termed *xenotropic*. Viruses capable of infecting cells of mice as well as other species are termed *polytropic*. The combinations of endogenous replication-competent MLVs and cell tropism factors are a reflection of selective breeding of mouse strains for susceptibility to various types of cancer.

Clinical Signs Mice were originally inbred for specific phenotypes, including mammary tumors and lymphomas. Thus, some strains of mice were genetically selected for unique combinations of endogenous MMTV and MLV in concert with susceptibility factors

that favored their expression and disease manifestations. In addition, noninfectious retroelements continue to reintegrate randomly within the genome during cell division as retro transposons. These ongoing integrations contribute to genetic drift, spontaneous mutations, and well-recognized mouse strain phenotypes, including the athymic nude allele, the hairless allele, and the rodless retina allele, among others.

Epizootiology Exogenous MMTV and MLV are horizontally transmissible, primarily through the milk of lactating females. Endogenous retroviruses and retroelements are inherited through the genome. Replication-competent endogenous MMTVs and MLVs are also transmissible like their exogenous counterparts, but differ by being integrated within the genome of the mouse.

Pathology Replication-competent MMTV and MLV, regardless of their exogenous or endogenous origin, are usually clinically silent. Their ability to cause neoplasia is a reflection of genetic selection for susceptibility factors that are genetically encoded within individual mouse strain genomes. MMTV derives its name from its association with induction of mammary carcinomas in mammary cancer-susceptible strains of mice. MLV is associated with lymphomas, the pattern of which is mouse strain specific. For example, AKR mice develop 100% prevalence of thymic lymphoma between 6 and 12 months of age, whereas aging BALB/c mice commonly develop multicentric lymphoma. In these strains of mice, multiple endogenous MLVs are coexpressed in tissues and undergo recombination events that allow them to target and transform cells into neoplasia. Despite its name, MMTV can induce lymphomas in some strains of mice, such as SJL mice which develop lymphomas arising from enteric lymphoid tissue and mesenteric lymph nodes.

Diagnosis Exogenous retroviruses have been eliminated from contemporary mouse populations, unless purposely introduced for experimental purposes. Because endogenous retroviruses and retroelements are encoded within the genome, and reflect the unique genetic composition of each strain of mouse, they are not targets of diagnostic pursuit.

Differential Diagnosis Patterns of some types of neoplasia within individual inbred strains of mice are a reflection of their endogenous retroviral integration.

Prevention and Control Exogenous retroviruses have been eliminated from laboratory mice by cesarean rederivation and foster nursing. MMTV-S, the 'Bittner agent', continues to be purposely maintained in some mouse breeding populations, but can be eliminated by foster-nursing or other means. Caution is advised when re-deriving such mouse colonies for other purposes, as elimination of exogenous MMTV will be an unintended consequence.

Research Complications Endogenous retroviruses and retroelements influence the life span of individual strains of mice, and random integrations during cell division can give rise to spontaneous mutations and genetic drift. It is estimated that significant mutations may arise due to mobile retroelement integrations every 50 generations.

p. Astrovirus Infection (Akkina, 2013; Yokoyama *et al.*, 2012, Farkas *et al.*, 2012)

Astroviruses are small, nonenveloped, single-stranded RNA viruses that have been associated with human gastroenteritis and detected in association with other enteric pathogens. The viral family Astroviridae is split into two genuses: *Avastrovirus* for those astroviruses infecting avians and *Mamastrovirus* for those infecting mammals. Astrovirus infection has been detected in research mice (MuAstV) using metagenomic analyses and appears to have a wide geographical, institutional, and host strain distribution.

Clinical Signs None reported.

Epizootiology PCR screening has found MuAstV infection in up to 22% of a variety of mouse strains housed in vendor and academic facilities in the United States and Japan. The virus has been detected most commonly in immunocompromised mice (NSG, NOD-SCID, NSG-3GS, C57BL6-Timp-3$^{-/-}$, and uPA-NOG), but also in immuncompetent strains (B6J, ICR, Bash2, and BALB/c). Both immunodeficient and immunocompetent mice are susceptible to MuAstV, but adaptive immunity is required to clear the virus. Based on human epidemiology indicating children are at highest risk for infection, the virus may preferentially infect young mice.

Pathology Immunodeficient mice showed no sign of pathology based on histopathology.

Diagnosis PCR data has indicated that MuAstV causes a systemic, chronic infection in immunocompromised mice, indicating samples from most tissues will be PCR positive. Yokoyama *et al.* (2012) detected high viral load (up to 10^9 genome copies) per fecal pellet from immunocompetent mice.

Differential Diagnosis None, in the absence of lesions and clinical disease.

Prevention and Control Because immunocompetent mice clear the infection, quarantine may be successful but lack of routine screening for MuAstV in laboratory mice will allow for uncontrolled spread of the infection.

Research Complications Based on limited surveys, MuAstV may have a high prevalence in laboratory mice. The impact of infection on both innate and adaptive immune responses warrants further investigation to assess the potential for confounding research data.

3. *Bacterial Diseases*

This section briefly describes the etiology, clinical signs, epizootiology, pathology, diagnosis, differential diagnoses, prevention and control, and research

complications of the most common bacterial diseases encountered in research colonies of mice. As sequencing technology becomes more available, the number and genus/species classification of bacteria potentially responsible for infections, in particular, opportunistic infections, will grow (Benga *et al.*, 2014). Potential candidates include members of Pasteurellacaeae, *Bordetella hinzii*, *Streptococcus danielae*, *Acinetobacter* spp., and others, for which little is currently known about their pathogenic potential.

a. *Lawsonia Intracellularis*

Etiology *Lawsonia intracellularis*, an obligate intracellular bacterium and the causative agent of proliferative enteropathy, is not a pathogen encountered in research colonies of mice but has been reported to infect wild mice and rats in close contact with infected livestock (Collins *et al.*, 2011).

Clinical Signs None reported but should consider *Lawsonia* as a differential in necropsy cases with gross or histologic evidence of proliferative lower bowel lesions.

Epizootiology Although mice are experimentally susceptible to infection and develop classic lesions of hyperplastic ileitis and typhlocolitis (Murakata *et al.*, 2008), susceptibility varied with mouse strain and source of inoculum from rabbits or swine, suggesting important differences in *L. intracellularis* strains.

Pathology Lawsonia infection may result in hyperplastic ileitis, typhlitis and/or colitis, and hemorrhagic intestines may be noted (Percy and Barthold, 2007).

Diagnosis *Lawsonia* spp. has been diagnosed using a variety of techniques, including PCR, immunohistochemistry, *in situ* hybridization, and Warthin–Starry silver stains.

Differential Diagnosis Bacterial infections associated with hyperplastic intestinal epithelium, including *C. rodentium* and enterohepatic helicobacter species in susceptible (typically immunodeficient) mouse strains.

Prevention and Control Species separation from hosts more commonly associated with natural infection (hamsters, ferrets, pigs).

Research Complications None reported.

b. Mycoplasmosis (Cassell *et al.*, 1986; Lindsey *et al.*, 1982, 1991i; Percy and Barthold, 2007)

The following section describes infection due to *Mycoplasma pulmonis* and summarizes infections associated with other murine mycoplasmas including *M. arthriditis*, *M. neurolyticum*, *M. collis*, and *M. muris*. Antigenic cross-reactivity among these species, and especially between *M. pulmonis* and *M. arthriditis*, mandates that reliable diagnostic strategies incremental to serology (ELISA, IFA, MFIA) such as culture (often false negative) and PCR be employed to distinguish potentially pathogenic infections. When screening cell lines for opportunistic pathogens, PCR is the most efficient method to discriminate between *M. pulmonis* and mycplasma contaminants associated with cell culture.

MYCOPLASMA PULMONIS

Etiology *M. 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; thus, subclinical infection is more 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 dyspnea, 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. 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 historically was a common infectious disease of mice, but improved housing, husbandry, and health surveillance have reduced its prevalence dramatically. Serologic data from a large diagnostic laboratory indicated *M. pulmonis* infection affects about 0.01% of conventionally housed mouse colonies in the United States and 0.16% in Europe (Pritchett-Corning *et al.*, 2009). *M. pulmonis* infection is contracted by inhalation and can occur in suckling and adult mice. Therefore, infection should be considered highly contagious. Mice injected with cells harvested from *M. pulmonis* contaminated cell cultures may develop disease. *M. pulmonis* can also be transmitted venerally; *in utero* infection has been demonstrated in rats but not in mice. Because transplacental infection occurs in rats, the same route may be possible in mice, particularly immunocompromised strains. Concomitant viral pneumonia (SV, mouse coronavirus) or elevated environmental ammonia concentrations may increase susceptibility to MRM. *M. pulmonis* also infects rats, hamsters, guinea pigs, and rabbits. Among these species, only rats are significant reservoirs of infection for mice.

Pathology *M. 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 infection of MRM is dose dependent. Doses of 10^4 colony-forming units (CFUs) or less cause mild, transient disease involving the upper respiratory tract and middle ears, whereas higher doses often lead to acute, lethal pneumonia. Additionally, *M. pulmonis* 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 other strains 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 (Fig. 3.34). 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 lumen. Chronic lung disease features suppurative bronchitis, bronchiolitis, and alveolitis (Fig. 3.35). Chronicity also increases the prevalence of bronchiectasis and abcessation.

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. Serology is sensitive but although antibodies do not clear the infection, seroconversion may be weak or take months and may not accurately differentiate between *M. pulmonis* infection and *M. arthriditis* infection (Cassell *et al.*, 1981). Therefore samples for culture and PCR of the upper respiratory tract should be obtained to confirm diagnosis. Buffered saline or *Mycoplasma* broth should be used to lavage the trachea, larynx, pharynx, and nasal passages. Specimens for culture from the genital tract are warranted if this site is suspected. *Mycoplasma* spp. 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 mycoplasma antigens in tissue sections or in cytological preparations of tracheobronchial or genital tract lavages (Brunnert *et al.*, 1994). PCR assays for *M. pulmonis* at veterinary diagnostic laboratories and PCR kits to screen cell culures for mycoplasma are readily available.

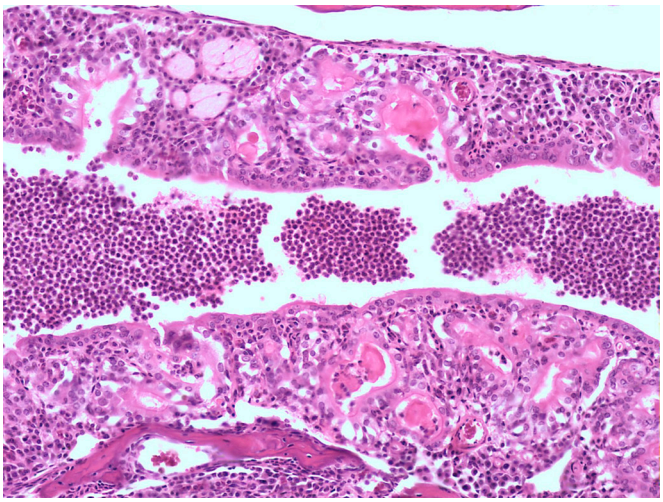

FIGURE 3.34 *Mycoplasma pulmonis*-induced rhinitis in a mouse. The turbinate mucosa contains accumulations of plasma cells and lymphocytes, the epithelium is decreased in thickness and has lost most cilia, and the lumen contains neutrophilic exudate. *Courtesy of Trenton Schoeb.*

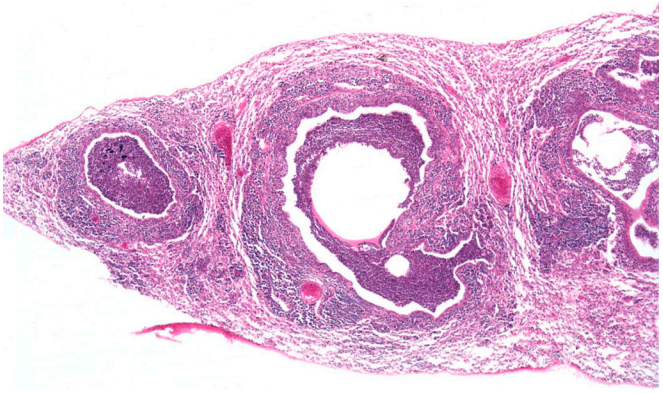

FIGURE 3.35 *Mycoplasma pulmonis*-induced bronchiolitis and bronchiolectasis in a mouse. The bronchioles are dilated, contain neutrophilic exudate, and are surrounded by accumulations of plasma cells and lymphocytes, and the mucosa is infiltrated with inflammatory cells. *Courtesy of Trenton Schoeb.*

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. SV also can cause bronchopneumonia in mice but can be detected by serology and immunohistochemistry. Other causes of respiratory infection include PVM, corynebacteriosis and, in immunodeficient mice, *Pneumocystis murina* infection. Combined infections with known pathogens or secondary opportunists also must be considered.

Prevention and Control Mice mount an effective immune response to *M. 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 clearance of the infection has been attributed to innate immune responses (Love *et al.*, 2010; Sun *et al.*, 2013). There is some evidence that antibody may facilitate phagocytosis of *M. pulmonis.* T-cell responses, however, appear to exacerbate *M. pulmonis* in mice, because immunity cannot be transferred with immune cells. In addition, athymic and neonatally thymectomized mice are not more susceptible than immunocompetent mice to *M. pulmonis* pneumonia. Nude and SCID mice develop less severe respiratory disease than immunocompetent mice but infection becomes systemic and they may develop suppurative disease in multiple organs and joints (arthritis).

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, PCR, and histopathology. Cesarean or embryo rederivation may eliminate infection, although vertical transmission may occur in immunocompromised mice. Treatment with tetracycline suppresses clinical disease but does not eliminate infection. Earlier interest in developing DNA-based vaccines against *M. pulmonis* has not achieved clinical application (Lai *et al.*, 1997).

Research Complications *M. 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. *M. pulmonis* may alter immunological responsiveness. For example, it is mitogenic for T and B lymphocytes and can increase NK cell activity. Perhaps one of the most important complications of *Mycoplasma* infection is contamination of cell lines and transplantable tumors.

Other Murine Mycoplasmas Cell lines are often contaminated with mycoplasma species such as *M. arginini*, *M. hyorhinis*, *M. orale*, or *M. fermentans* that can distort the results of *in vitro* assays (Garner *et al.*, 2000). Initial evidence of a contamination is often by PCR evidence of mycoplasma at the genus level when cell lines are PCR screened for opportunistic murine pathogens prior to use in mice. Other than *M. pulmonis*, these mycoplasmas are not normally considered mouse pathogens in immunocompetent mice. In contrast, injection of mycoplasma contaminated cells into immunodeficient mice (e.g., xenografts) may result in clinical disease or confounding effects on immune responses (Peterson, 2008). Mycoplasma contamination of murine embryonic stem cells has adversely affected germline transmission and postnatal health of chimeric progeny (Markoullis *et al.*, 2009).

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 mice 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. neurolyticum* 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 h of rolling, animals become comatose and usually die within 4 h. All published reports of rolling disease are associated with experimental inoculation of *M. neurolyticum* 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. neurolyticum* 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*- and *P. pneumotropica*-caused otitis. *M. pulmonis* has been recovered from the brain of mice but does not seem to cause overt neurological disease.

Hemotropic Mycoplasmas Ribosomal RNA sequencing has reclassified *Hemobartonella muris* and *Eperythrozoon coccoides* as *Mycoplasma hemomuris* and *Mycoplasma coccoides*, respectively (Neimark *et al.*, 2005; Percy and Barthold, 2007). Distinct from the mycoplasmas just discussed, these agents are trophic for red blood cells and cause anemia and hemolytic disease. These

infections could be encountered in wild mice but are rarely found in research mice. Diagnosis is by morphologic assessment of blood smears and PCR.

Clinical Signs Mice infected with *M. 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. Hemotropic mycoplasma infections are long-lived and are expressed clinically in one of two ways: acute febrile anemia and latent or subclinical infection that can be reactivated by splenectomy. The carrier state may be lifelong.

Epizootiology The primary natural vector of *M. coccoides*, historically, is the mouse louse, *Polyplax serrata.* 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, *M. 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 of detecting *M. coccoides* infection. These procedures provoke mycoplasmemia, usually within 2–4 days. Because mycoplasmemia may be transient, blood smears stained by the Romanowsky or indirect immunofluorescence procedures of the blood should be prepared every 6 h, beginning at 48 h after splenectomy of index animals or inoculation of test specimens into splenectomized animals to ensure that mycoplasmemia is not missed.

Prevention and Control Treatment of *M. coccoides* infection is not practical. Control is based on culling 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 screened for mycoplasma contamination by inoculation of splenectomized mice.

Research Complications Subclinical infection can be reactivated by irradiation, immunosuppressive therapy, or intercurrent disease. Conversely, *M. 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.

c. CAR Bacillus Infection

Etiology CAR bacillus is a slender, gram-negative, non-spore-forming bacillus, which, in rats, produces clinical disease and lesions that closely resemble those of MRM (see Chapter 4).

Clinical Signs Chronic respiratory disease has been produced in mice by experimental inoculation, but natural clinical disease is rare (Griffith *et al.*, 1988; Pritchett-Corning *et al.*, 2009). Furthermore, putative natural cases were reported in mice that were seropositive for SV and pneumonia virus of mice. Therefore, CAR bacillus may exacerbate respiratory disease as an opportunist rather than as a primary pathogen. On balance, it is assumed that mice contract natural infection, but attributing severe chronic respiratory disease in mice solely to CAR bacillus should be supported by screening for other respiratory pathogens.

Epizootiology CAR bacillus is transmitted by direct contact; dirty bedding transfer to sentinel mice may not reflect colony infection status.

Pathology Lung lesions are typically mild in mice and are similar to respiratory mycoplasmosis. Uncomplicated CAR bacillus infection results in peribronchiole cuffing with lymphocytes and plasma cells. Severe bronchiolitis and pneumonia are possible (Fig. 3.36). Fatal bronchopneumonia was reported in OB/OB mice (Griffith *et al.*, 1988).

Diagnosis An ELISA for serological screening is routinely used; PCR and histology are used for definitive diagnosis. In active infection, histologic assessment using Warthin–Starry or similar stains will reveal argyrophilic bacilli adherent to the apical membranes of bronchial respiratory epithelium along with the presence of peribronchial lymphocytes (Fig. 3.37). Alternatively, immunohistochemistry assays have also been used successfully to detect infection. Recovery of CAR bacillus requires cell culture or culture in embryonated eggs.

Differential Diagnosis Respiratory mycoplasmosis, Bordetella (avium, hinzii).

Prevention and Control Given CAR bacillus does not form spores, disinfection of the environment should be effective. Treatment using sulfamerazine (500 mg/l)

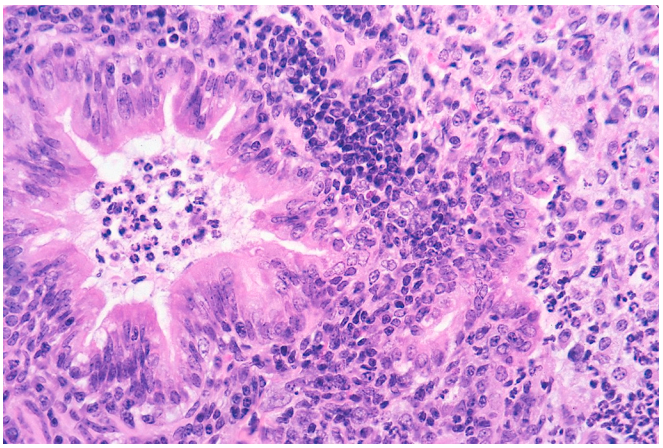

FIGURE 3.36 CAR bacillus-induced bronchiolitis and pneumonia in a mouse. The bronchiole is surrounded by lymphocytes, the lumen contains neutrophilic exudate, and the epithelium is hyperplastic. Adjacent alveoli contain neutrophils and macrophages. *Courtesy of Trenton Schoeb.*

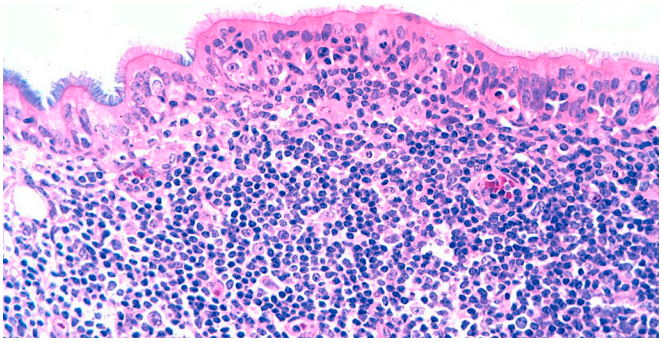

FIGURE 3.37 CAR bacillus-induced rhinitis in a mouse. The epithelium is infiltrated by neutrophils and lymphocytes, and the underlying lymphoid tissue is hyperplastic. Basophilic CAR bacilli are visible among the cilia at left. *Courtesy of Trenton Schoeb.*

in drinking water may eradicate infection (Matsushita and Suzuki, 1995) but culling or embryo rederivation is recommended.

Research Complications Infection is most often subclinical, but like other infectious agents for mice, may confound studies particularly when mice are immunocompromised (Griffith *et al.*, 1988).

d. Transmissible Murine Colonic Hyperplasia

Etiology The causative agent of transmissible murine colonic hyperplasia, *C. 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 *C. rodentium* infection can be a self-limiting colitis with sterilizing immunity or lead to severe colitis with life-threatening dehydration. 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 *C. rodentium* is not detected in the gastrointestinal flora of normal mice, and therefore, there is not a carrier state. It is thought to be introduced by contaminated mice, food, or bedding, from which it spreads by contact or additional fecal contamination. *C. rodentium* shares several pathogenic mechanisms, such as attaching and effacing lesions mediated by the intimin receptor, with select *Escherichia coli* (reviewed in Collins *et al.* (2014)). *C. rodentium* is used experimentally to model colitis caused by enteropathogenic (EPEC) and enterohemorrhagic *E. coli* (EHEC) in humans (Mallick *et al.*, 2012; Collins *et al.*, 2014). 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. Interestingly, C57BL mice obtained from different commercial sources have varying susceptibility to *C. rodentium* (ostensibly due to the presence or absence of segmented filamentous bacteria). Diet also can modulate infection, but specific dietary factors responsible for this effect have not been identified.

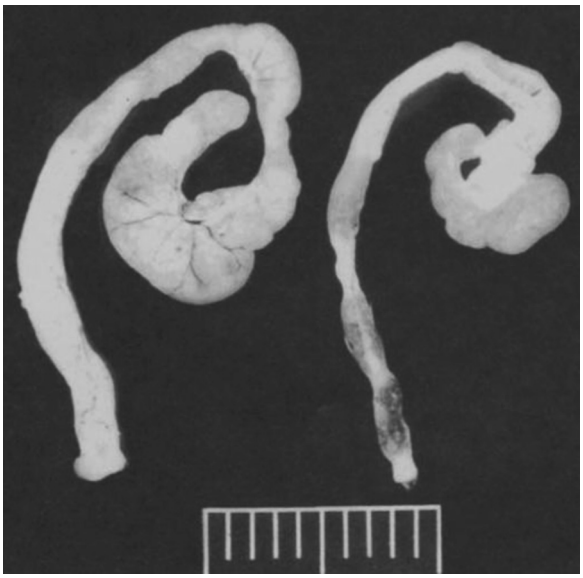

FIGURE 3.38 Colons of a normal mouse (right) and of a mouse with transmissible murine colonic hyperplasia (left). The descending colon is thickened and opaque because of mucosal hyperplasia. *From Barthold et al.* (1978).

Pathology *C. 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 and Falkow, 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. 3.38). Affected colon 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. 3.39). Lesions subside after several weeks. Intestinal 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 can be cultured on MacConkey's agar during early phases of infection, whereas the intestine may be free of *C. rodentium* during later stages of the disease. *C. rodentium* also can be detected by molecular hybridization (Schauer *et al.*, 1995).

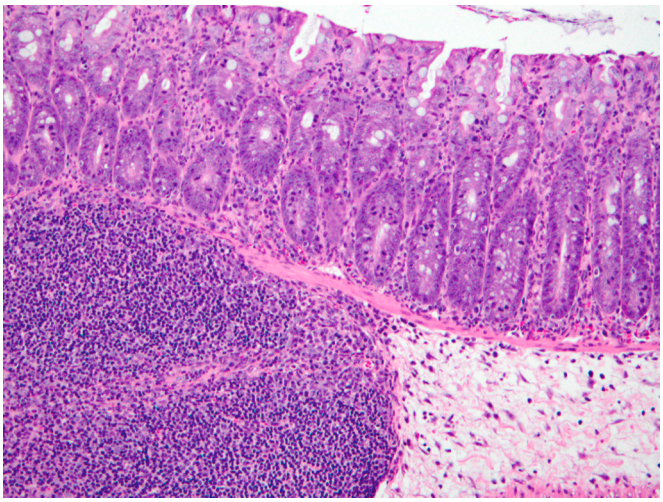

FIGURE 3.39 Colonic inflammation, edema, mild hyperplasia of the epithelium, and significant development of mucosa-associated lymphoid tissue (MALT) caused by *C. rodentium* infection. *Courtesy of Suresh Muthupalani.*

Differential Diagnosis Transmissible murine colonic hyperplasia must be differentiated from other diarrheal diseases of mice, including infections caused by coronavirus, rotavirus, adenovirus, reovirus, *Salmonella*, *C. 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. rodentium* may contaminate food, bedding, or water, proper disinfection of such materials is prudent before they are used for susceptible animals. Additionally, the employment of microbarrier caging can reduce transmission. Surveillance for *C. rodentium* should be incorporated into quality-assurance programs, and the organism screened for during quarantine of incoming mice from atypical sources.

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). *C. rodentium* 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). Immunocompromised mice infected with *C. rodentium* will die from sepsis.

e. Pseudomoniasis (Lindsey *et al.*, 1991b; Percy and Barthold, 2007)

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

Clinical Signs *P. aeruginosa* infections are almost always silent, but immunologically compromised animals are prone to septicemia (Brownstein, 1978). *P. aeruginosa* can, e.g., cause severe or lethal infections in athymic and SCID 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, especially neutropenia. Neurologic signs are rare, but there are reports of central nervous system infection. Chronic proliferative inflammation in the cochlea and vestibular apparatus with dissolution of surrounding bone may cause torticollis.

Epizootiology *P. aeruginosa* is not considered a component 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 the 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 or recent immunosuppression), 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 h and then culturing the sipper tubes. *P. aeruginosa* can also be cultured from feces.

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. *P. aeruginosa* has a long history in the literature of antibiotic resistance and resistance to quaternary amine disinfectants.

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

f. Pasteurella Pneumotropica Infection (Lindsey *et al.*, 1991a; Percy and Barthold, 2007)

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 subclinical. 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 SV. It has also 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 *P. 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 abcessation. 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 (Fig. 3.40). 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.

FIGURE 3.40 Multiple abscesses (head, orbita) in a nude mouse caused by *P. pneumotropica*.

Diagnosis Diagnosis requires isolation of the organism on standard bacteriological media. Although infection can be detected serologically by ELISA (Wullenweber-Schmidt *et al.*, 1988; Boot *et al.*, 1995a, b), subclinical carriers often do not seroconvert. PCR assays also are available (Dole *et al.*, 2010) and have shown that *P. pneumotropica* did not transmit from infected mice to contact or dirty bedding sentinels (Ouellet *et al.*, 2011; Dole *et al.*, 2013).

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

Treatment Antibiotic sensitivity testing *in vitro* indicated *P. pneumotropica* was significantly more sensitive than *P. aeruginosa* to enrofloxacin (Sasaki *et al.*, 2007). Enrofloxacin in the drinking water at 85 mg/kg daily for 7 days eliminated clinical signs and infection in a closed breeding colonic of transgenic mice and after 14 days of treatment there were no detectable carriers when the colony was screened 4 weeks later (Matsumiya and Lavoie, 2003).

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.

Research Complications Clinically severe infection in immunodeficient mice is the major complication. Although clinically silent, experimental evidence has shown that *P. pneumotropica* infection in immunocompetent mice (C57BL/6) stimulated transcription of multiple proinflammatory cytokines for at least 7 days with residual elevation detectable 28 days later (Patten *et al.*, 2010).

g. Helicobacteriosis

Pioneering studies conducted in the 1990s first linked a novel microaerobic bacterium, *Helicobacter hepaticus*, with chronic active hepatitis and hepatic tumors in A/JCr mice (Fox *et al.*, 1994, 2011; Ward *et al.*, 1994). The organism could be visualized by electron microscopy in the bile canaliculi of the liver in susceptible mouse strains (Fig. 3.41). Subsequently, it was associated with inflammatory bowel disease in several murine models (Table 3.13) which were further developed to examine the role of immune cell subsets, such as T regulatory cells, in the pathogenesis of inflammatory bowel disease (IBD) and colon cancer (Fig. 3.42). Helicobacteriosis is

now appreciated to be a common infection of laboratory mice. It is caused by a growing list of *Helicobacter* spp. that vary in clinical, pathologic, and epidemiologic significance (Whary and Fox, 2004; Fox *et al.*, 2011). Because recognition and investigation of helicobacteriosis continues to evolve, many important questions about the impact of this infection on mice remain unresolved.

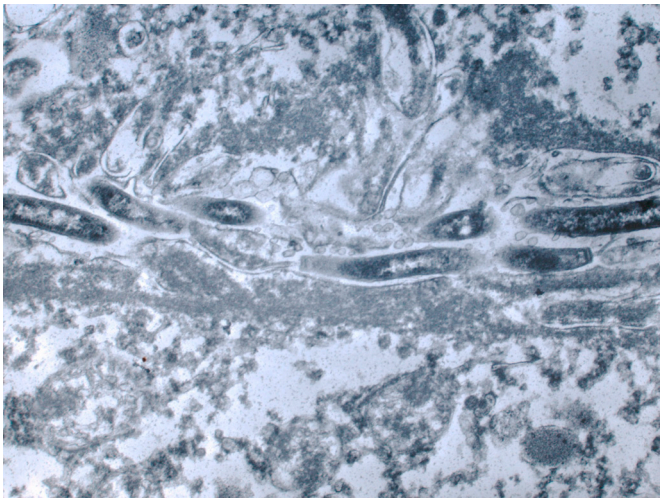

FIGURE 3.41 Electron micrograph of *H. hepaticus* in the hepatic biliary canaliculi of an SCID mouse.

H. 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. (EHS) (Fox *et al.*, 1994, 2011; Ward *et al.*, 1994; Suerbaum *et al.*, 2003). However, current information about other murine helicobacters is summarized in the concluding section.

Etiology *Helicobacter* spp. are gram-negative, microaerophilic, curved to spiral-shaped organisms that have been isolated from the gastrointestinal mucosa of many mammals, including humans and mice (Fox *et al.*, 2000; Whary and Fox, 2004). To date, the genus includes 20 formally named *Helicobacter* spp. assigned on the basis of 16S rRNA analysis, complemented by biochemical, molecular, and morphological characteristics. 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, 80% N_2, 10% H_2).

There are currently 11 formally named *Helicobacter* species have been isolated from laboratory mice, as well as several other novel *Helicobacter* spp. awaiting formal naming. Species isolated from mice include *H. hepaticus*, *H. bilis* (which also infects rats), *H. muridarum*, *H. rappini*, and *H. rodentium*, *H. ganmani*, *H. mastomyrinus*, *H. magdeburgensis, and H. typhlonius*, each of which

TABLE 3.13 *H. hepaticus*- and *H. bilis*-Associated IBD and Colon Cancer in Mice[a]

Genetic status of mice	Type of defect	Pathology	References
CD45RB (high)-reconstituted ICR defined flora *scids*	Reconstitution with naïve $CD4^+$ T cells	Typhlocolitis	Cahill *et al.* (1997)
TCRα, β mutants	Defective T-receptors	Typhlocolitis	Chin *et al.* (2000)
Scid ICR-defined flora[b]	T- and B-cell deficient	Typhlocolitis	Shomer *et al.* (1998), Shomer *et al.* (1997)
C57BL/IL-$10^{-/-}$[c]	Lacks IL-10	Typhlocolitis	Burich *et al.* (2001), Kullberg *et al.* (2001), Kullberg *et al.* (2006), Kullberg *et al.* (2002)
C57BLRag$2^{-/-}$	T- and B-cell deficient	Typhlocolitis	Burich *et al.* (2001)
129SvEv/Rag$2^{-/-}$	T- and B-cell deficient	Typhlocolitis, colon cancer	Erdman *et al.* (2003a, 2009), Knutson *et al.* (2013), Mangerich *et al.* (2012)
IL-$7^{-/-}$/RAG-$2^{-/-}$	IL7, T and B cell deficiency	None	von Freeden-Jeffry *et al.* (1998)
A/JCr	Normal	Typhlitis	Fox *et al.* (1996a)
Swiss Webster gnotobiotic	Monoassociated	Enterocolitis	Fox *et al.* (1996b)
129SvEv/NF-κβ ($p50^{-/-}p65^{+/-}$)	Defective NF-κβ pathway	Typhlocolitis	Erdman *et al.* (2001)
mdrla$^{-/-}$[d]	Lack P-glycoprotein	Typhlocolitis	Maggio-Price *et al.* (2002)
SMAD3$^{-/-}$[d]	Defective TGF-β pathway	Typhlocolitis, colon cancer	Maggio-Price *et al.* (2006)

[a]*In mice of the same genetic status which had* H. hepaticus *(or other* Helicobacter *spp.)-negative microflora, no intestinal disease was noted.*
[b]*Mice infected with* H. bilis *also developed IBD (Shomer* et al., *1997).*
[c]*IBD also developed in C57Bl/IL-*$10^{-/-}$ *mice experimentally infected with a novel urease-negative* Helicobacter *spp. (Fox* et al., *1999) now named* H. typhlonius *(Franklin* et al., *2001); also IBD produced with* H. trogontum *(Whary* et al., *2006) and* H. cinaedi *(Shen* et al., *2009).*
[d]H. bilis *produces IBD (Maggio-Price* et al., *2002, 2006) and colon cancer (Maggio-Price* et al., *2006).*

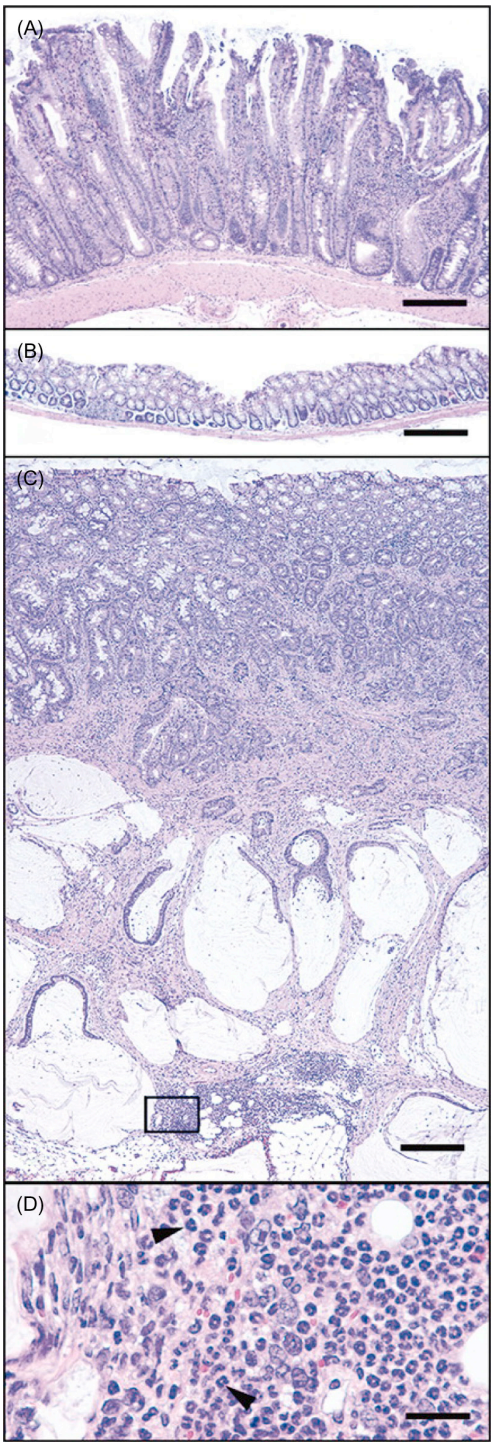

FIGURE 3.42 Regulatory cells lacking IL-10 did not suppress inflammation or dysplasia when transferred either before or after *H. hepaticus* infection. (A) Infected untreated mice developed moderate to severe inflammation, hyperplasia, and dysplasia in the cecum and colon at 4 months after infection. (B) Inflammation and dysplasia were significantly suppressed after transfer of wild-type regulatory cells. (C) Regulatory cells lacking IL-10 were unable to suppress inflammation or dysplasia. Mice receiving IL-$10^{-/-}$ regulatory cells had an increased frequency of mucinous cancer. (D) Dense inflammatory infiltrate in the mucinous tumor (inset of C) composed mainly of neutrophils (arrowheads) and a few macrophages. A–C: bar = 250 µm; D: bar = 25 µm.

have been formally named (except for *H. rappini*) (Fox and Lee, 1997; Franklin *et al.*, 1999; Whary and Fox, 2004). Most recently, *Helicobacter pullorum*, a human pathogen, has been isolated from commercial, barrier-maintained mice (Boutin *et al.*, 2010). These EHS 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/JCr mice (Fox *et al.*, 1996a). Infection of immune-dysregulated mice with *H. hepaticus* can cause inflammatory bowel disease, which may present as rectal prolapse and/or diarrhea (Miller *et al.*, 2014).

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.*, 1998b; Taylor *et al.*, 2007; Lofgren *et al.*, 2011). 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. *H. hepaticus* and *H. bilis*, e.g., can cause inflammation in the gastrointestinal tract, which is expressed as IBD and colon cancer in immunodeficient mice or typhlitis in A/JCr mice infected with *H. hepaticus* (Ward *et al.*, 1996; Knutson *et al.*, 2013; Shomer *et al.*, 1997; Erdman *et al.*, 2003b; Nguyen *et al.*, 2013). Thickening of the cecum and large bowel develops because of proliferative typhlitis, colitis, proctitis, and lower bowel carcinoma. These lesions can occur without coincident hepatitis. Indeed, *Helicobacter* spp. induced IBD and colon carcinoma are increasingly popular models to study pathogenesis of the disease in humans (Table 3.13).

Helicobacter spp. also can cause liver disease. Bacterial translocation is thought to occur and results in colonization of the liver and progressive hepatitis. It is characterized by angiocentric nonsuppurative hepatitis and hepatic necrosis (Fig. 3.43). 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 in bile canaliculi 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.*, 1996a). This lesion may

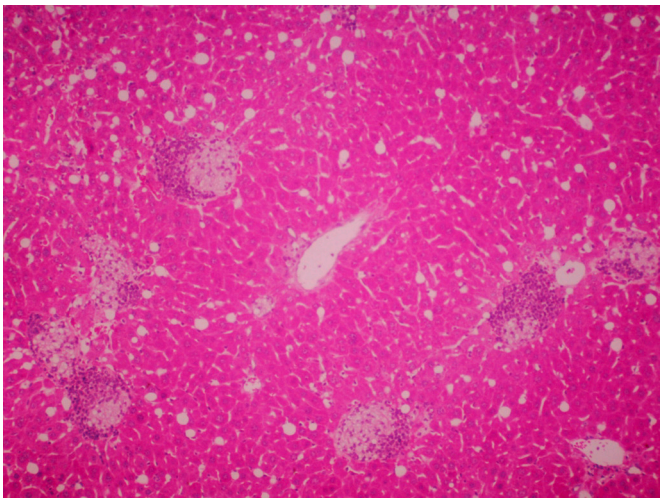

FIGURE 3.43 *H. bilis*-induced nonsuppurative hepatitis and hepatic necrosis. Inflammation originates in portal triads and spreads to adjacent hepatic parenchyma.

increase susceptibility to hepatomas and hepatocellular carcinomas among aged male A/JCr and B6C3F1 mice from infected colonies. An increased incidence of hepatic hemangiosarcoma 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. Studies with *H. hepaticus* in recombinant inbred mice also indicate that disease susceptibility has multigenetic properties (Hailey *et al.*, 1998; Fox and Lee, 1997; Ihrig *et al.*, 1999; Franklin, 2006; Hillhouse *et al.*, 2011).

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 (Fox *et al.*, 1998a; Shames *et al.*, 1995; Beckwith *et al.*, 1997). However, genus-specific PCR does not differentiate among different *Helicobacter* spp. Molecular speciation can be accomplished by 16S rRNA sequencing, restriction fragment length polymorphism analysis of the PCR product or use of species-specific PCR assays. This procedure requires suitable skill and experience to avoid technological pitfalls and should be performed by qualified laboratories. An IgG ELISA using the outer membrane protein as the antigen has been proposed for serological diagnosis, but shared antigens among EHS create lack of specificity for the assay. As noted above, helicobacters can be isolated on antibiotic-impregnated blood agar under microaerobic conditions and can then be speciated biochemically, and by *Helicobacter* species-specific PCR. Isolation of *H. hepaticus* and from other *Helicobacter* spp. with spiral to curved morphology from feces should be preceded by passing slurried samples through a 0.45-μm filter before plating. If infection with larger fusiform 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 (Whary and Fox, 2004).

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. *C. rodentium* also causes colonic hyperplasia, which can present as rectal prolapse.

Infections Caused by Other Helicobacters of Mice *H. bilis* has been isolated from the livers and intestines of aged mice and experimentally induces IBD in SCID mice as does *H. hepaticus*. *H. bilis* also experimentally produces lower bowel cancer in immunocompromised mice (Nguyen *et al.*, 2013). *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. *H. 'rappini'* has been isolated from the feces of mice without clinical signs. *H. 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 IL-10$^{-/-}$ and SCID mice (Franklin *et al.*, 1999, 2001; Fox *et al.*, 1999). Decreased reproductive efficiency has been reported in IL10 knockout mice infected with *H. rodentium* and/or *H. typhlonius* (Sharp *et al.*, 2008).

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, metronidazole, and bismuth given for 2 weeks (Del Carmen Martino-Cardona *et al.*, 2010). This strategy requires repeated daily gavage rather than administration in drinking water, but it has successfully eliminated *H. hepaticus* from naturally infected mice. Antibiotic impregnated wafers have been used to eradicate *Helicobacter* spp. in mouse colonies (Kerton and Warden, 2006). 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. In 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.*, 2011). *H. hepaticus* infections provoke a strong Th1 proinflammatory response, which may perturb other immunological responses. *H. hepaticus* infection also has been incriminated as a cofactor or promoter in the development of hepatic neoplasia in A/JCr, B6C3F1, AB6F1, B6AF1, and CARKO mice (Hailey *et al.*, 1998; Fox *et al.*, 1998a; Garcia *et al.*, 2008, 2011).

h. Salmonellosis (Ganaway, 1982; Lindsey *et al.*, 1991c; Percy and Barthold, 2007)

Etiology The genus *Salmonella* contains two species, *S. bongori* which infects mainly poikilotherms and rarely, humans, and *S. enterica* which includes approximately 2500 serovars and are a major cause of food-borne illness in humans (Fookes *et al.*, 2011). The salmonella of historical importance in mice that are now rare include *S. enterica* subsp. *enterica* serovar Typhimurium (aka *S. Typhimurium*) and serovar Enteritidis (*S. Enteritidis*). *S. Enteritidis* is a motile, gram-negative rod that rarely ferments lactose. The genomes of many strains have been sequenced. Virulence factors carried on pathogenicity islands and plasmids include antimicrobial resistance genes, type III secretion systems, Vi antigen, lipopolysaccharide and other surface polysaccharides, flagella, and factors essential for a intracellular life cycle in macrophages (de Jong *et al.*, 2012). Pathogen-associated molecular patterns (PAMPs) unique to salmonella interact with TLRs and NOD-like receptors (NLRs) which recruit neutrophils and macrophages leading to inflammasome formation and release of pro-inflammatory IL-6, IL-1β, TNF-α, and IFN-γ.

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 episodic 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 *S. Typhimurium* is commonly used experimentally and cross-contamination in a mouse facility is a risk. 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. Salmonella have a broad host range and 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 humans.

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 subclinical 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 h 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. *S. 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, whereas 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, 2007).

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. Subclinically infected animals can be detected by fecal culture using selective enrichment media (Selenite F broth plus cystine followed by streaking on brilliant green agar). 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. PCR-based assays are also available.

Differential Diagnosis Salmonellosis must be differentiated from other bacterial diseases, including Tyzzer's disease, *Helicobacter* spp., pseudomoniasis, corynebacteriosis, *C. rodentium*, 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 personnel working with them.

i. Streptobacillosis (Lindsey *et al.*, 1991e; Percy and Barthold, 2007)

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 rats and mice, but modern husbandry, production, and health surveillance strategies have reduced its impact dramatically (Wullenweber, 1995). Subclinical, persistently infected rats are the most likely source of dissemination to mice, but mouse-to-mouse transmission then ensues. Transmission may occur from aerogenic exposure, bite wounds, or contaminated equipment, feed, or bedding. *S. moniliformis* is also pathogenic for humans, causing rat bite fever (Haverhill fever).

Pathology During acute disease, necrotic lesions develop in thoracic and abdominal viscera, especially in the 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 on blood agar. The organism has been recovered from joint fluid as long as 26 months after infection. Isolation from chronic lesions requires serum-enriched medium. *S. moniliformis* as a cause of septic joints in humans has been diagnosed using PCR and electrospray-ionization followed by mass spectrometry (Mackey *et al.*, 2014).

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; Percy and Barthold, 2007)

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; Dole *et al.*, 2013).

Clinical Signs *C. kutscheri* infection is often subclinical 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 inappetence, emaciation, rough hair coat, hunched posture, hyperpnea, nasal and ocular discharge, cutaneous ulceration, and arthritis. *C. bovis* infection causes hyperkeratotic dermatitis characterized by scaly skin, which is accompanied by alopecia in haired mice. Severe infection may cause death. Corynebacterial keratoconjunctivitis has been reported in aged C57BL/6 mice (McWilliams *et al.*, 1993).

Epizootiology Subclinically infected animals harbor *C. kutscheri* in the upper alimentary tract, colon, respiratory tract, regional lymph nodes, middle ear, and preputial gland. *C. bovis* colonizes skin and is shed in feces. Therefore, transmission is by direct contact, fecal–oral contact, and aerosol. 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.

Pathology Lesions caused by *C. kutscheri* develop from hematogenous spread to various internal organs and appear as gray–white nodules in the kidney, liver, lung, and other sites. Cervical lymphadenopathy and arthritis of the carpometacarpal and tarsometatarsal joints also may occur. Septic, necrotic lesions often contain caseous material or liquefied exudate. Histologic lesions are characterized by coagulative or caseous necrosis bordered by intense neutrophilic infiltration. Colonies of gram-positive organisms with 'Chinese letter' configurations can usually be demonstrated using tissue Gram stains of 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 pan arthritis. *C. 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.

FIGURE 3.44 Hyperkeratosis associated with *C. bovis* infection.

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. 3.44). Hyperkeratosis is typically more severe in glabrous athymic mice than in haired mice. Organisms can be demonstrated in hyperkeratotic layers by Gram stain.

Diagnosis *C. kutscheri* is usually diagnosed by culture and 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.*, 1995a). PCR of skin swabs or feces is a sensitive and specific method for the detection of *C. bovis* infection in mice (Dole *et al.*, 2013).

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. Diffuse scaling dermatitis in athymic nude mice is classic for *C. bovis* infection; however, in one case report *Staphylococcus xylosus* was instead isolated in high numbers from the skin lesions (Russo *et al.*, 2013). Hyperkeratotic dermatitis caused by

C. bovis must be differentiated from scaly skin caused by low humidity in glabrous mice.

Prevention and Control *C. kutscheri* infection occurs sporadically and infected colonies should be culled or rederived into an SPF facility as treatment is not curative and control is difficult.

C. bovis can be endemic in athymic nude mouse colonies. Prevention and control are difficult because both immunocompetent and athymic mice as well as humans can carry *C. bovis* on the skin and in the upper respiratory system, respectively. *C. bovis* readily contaminates the environment as aerosolization within a class II biosafety cabinet was shown to spread the bacterium during cage-change procedures (Burr *et al.*, 2012). Antibiotic treatment has been unrewarding (Burr *et al.*, 2011)

Research Complications Corynebacteriosis can cause 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 (Lindsey *et al.*, 1991d; Shimizu, 1994; Percy and Barthold, 2007; Besch-Williford and Franklin, 2007)

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 profiles. Pathogenic staphylococci are typically coagulase-positive, although *S. xylosus* has caused serious infections and is coagulase-negative (Gozalo *et al.*, 2010).

Clinical Signs Staphylococcosis causes suppurative conjunctivitis, periorbital and retroorbital abscesses, preputial adenitis, and pyoderma in mice, particularly in immunocompromised strains such as nude 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. SJL mice, which are NK cell deficient, are prone to necrotic dermatitis on the tail secondary to *S. xylosus* infection.

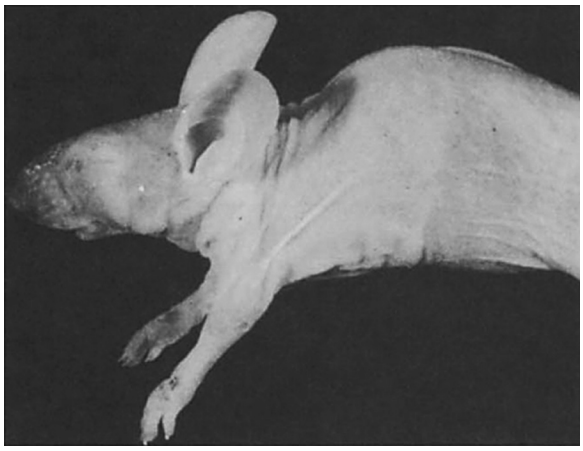

FIGURE 3.45 Furunculosis in an athymic mouse.

Epizootiology Staphylococci are ubiquitous and can be carried on the skin and in the nasopharynx 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 are among 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. 3.45). 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, ulcerative and necrotic dermatitis involving the head and neck but may extend to the shoulders and forelegs (Percy and Barthold, 2007). Superficial or deep abscesses may occur in conjunction with dermatitis or separately, as, e.g., in the external male genitalia. Histologically, acute skin infections result in ulceration with neutrophils in the dermis and subcutis. Chronic lesions contain lymphocytes, macrophages, and fibroblasts. Deep infections appear as coalescing botryomycotic pyogranulomas with necrotic centers containing bacterial colonies. Infected athymic mice may develop

furunculosis of the muzzle and face accompanied by regional lymphadenitis.

Diagnosis Diagnosis is made by documenting gross and histological lesions, including Gram staining of suspect tissues, complemented by isolation of gram-positive, coagulase-positive (*S. aureus*), or coagulase-negative *Staphylococcus* species.

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. In affected animals, nail trimming can 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.

l. Streptococcosis (Lindsey *et al.*, 1991f; Nakayama and Weyant, 1994; Percy and Barthold, 2007; Besch-Williford and Franklin, 2007)

Etiology Streptococci are ubiquitous commensal gram-positive organisms and in some cases, primary pathogens. Pathogenic streptococcal infections in laboratory mice are caused by β-hemolytic organisms in Lancefield's group C, but epizootics caused by group A streptococci have occurred, and group G organisms have been isolated occasionally. Group D has been reclassified as an *Enterococcus*. Alpha-hemolytic streptococci can cause systemic disease in SCID mice, and group B *Streptococcus* sp. infection has been reported to cause meningoencephalitis in athymic mice (Schenkman *et al.*, 1994). Additionally, *Streptococcus dysgalactiae* subsp. *equisimilis* has Lancefield group G or C antigens and was isolated from visceral abscesses of immunocompetent mice (Greenstein *et al.*, 1994).

Clinical Signs 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.

Epizootiology Mice can carry streptococci subclinically 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.

Pathology Systemic lesions reflect hematogenous dissemination and include abscessation, endocarditis, splenomegaly, and lymphadenopathy (Percy and Barthold, 2007). 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.

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

Differential Diagnosis Streptococcosis must be differentiated from other suppurative infections of mice, including staphylococcosis, pasteurellosis, corynebacteriosis, and pseudomoniasis.

Prevention and Control Removal of affected animals, sterilization of food and bedding, and frequent changing of bedding may limit or reduce transmission.

Research Complications Immunodeficient mice are at increased risk for streptococcosis.

m. Colibacillosis (Percy and Barthold, 2007)

Etiology *E. coli* is a small gram-negative rod that is a normal inhabitant of the mouse intestine.

Epizootiology 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 *E. coli* (Waggie *et al.*, 1988; Arthur *et al.*, 2012).

Clinical Signs Affected mice develop lethargy and fecal staining.

Pathology 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 Diagnosis depends on demonstrating lesions and isolating non-lactose-fermenting *E. coli.*

Differential Diagnosis This condition must be differentiated from proliferative and inflammatory intestinal disease caused by *Lawsonia intracellularis*, *C. rodentium*, or 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.

Prevention and Control Removal of affected animals and disinfection of caging and equipment will limit or reduce transmission.

Research Complications Clinical illness may develop in immunodeficient mice.

n. Klebsiellosis

Historically, *Klebsiella pneumoniae* is a ubiquitous gram-negative organism that is a natural inhabitant of the mouse alimentary tract. Most commercial vendors have excluded it from their barriers. 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.

Etiology *Klebsiella oxytoca* is an opportunistic pathogen implicated in various clinical diseases in animals and humans.

Epizootiology *K. oxytoca* also is purported to be an etiological agent of antibiotic-associated hemorrhagic colitis (AAHC) in adult humans and adolescents. In animals, *K. oxytoca* has been isolated from apparently healthy sentinel rodents being monitored for pathogens in health surveillance programs and from utero-ovarian infections including suppurative endometritis, salpingitis, perioophoritis, and peritonitis in aged B6C3F1 mice (Davis *et al.*, 1987; Rao *et al.*, 1987). A model of AAHC has been developed in rats by administering amoxicillin-clavulanate followed by orally infecting rats with a strain of *K. oxytoca* cultured from a patient with AAHC. Studies in humans suggest that *K. oxytoca* exerts its pathogenicity in part through a cytotoxin. Recently, authors have showed that several animal isolates of *K. oxytoca*, including clinical isolates, produced secreted products in bacterial culture supernatant that display cytotoxicity on HEp-2 and HeLa cells, indicating the ability to produce cytotoxin. Using mass spectroscopy techniques, they also confirmed tilivalline as the cytotoxin present in animal *K. oxytoca* strains. Tilivalline may serve as a biomarker for *K. oxytoca*-induced cytotoxicity (Darby *et al.*, 2014).

Clinical Signs *K. oxytoca* has been cultured from cases of suppurative otitis media, urogenital tract infections, and pneumonia in C3H/HeJ and NMRI-Foxn1 (*nu*) mice (Bleich *et al.*, 2008). Additionally, *K. oxytoca* was recently cultured from three breeding colonies of NOD. Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (NSG) mice with chronic renal inflammation and ascending urinary tract infections (Foreman *et al.*, 2011).

Differential Diagnosis Other bacterial infections capable of causing suppurative lesions, including staphylococci, streptococci, *Pasteurella* sp., and *E. coli*, among others are considered a differential diagnosis.

Research Complications Morbidity and mortality from spontaneous infections can affect ongoing research.

o. Clostridium Infections

CLOSTRIDIUM DIFFICILE

Etiology *Clostridium difficile* was identified as the etiology of antimicrobial-associated pseudomembranous colitis in humans and currently a considerable cause of morbidity in hospitalized patients who acquire nosocomial infections. In the early 2000s, an increased interest in *C. difficile* infection (CDI) resulted from the emergence of a hyper-virulent strain (NAP1/BI/027) associated with frequent recurrences and more severe clinical disease (Abou Chakra *et al.*, 2014; McFarland, 2009; Kuijper *et al.*, 2006). *C. difficile* has also been implicated in antibiotic-associated colitis in Syrian hamsters (Bartlett *et al.*, 1977), guinea pigs (Lowe *et al.*, 1980), rabbits (Thilsted *et al.*, 1981; Ryden *et al.*, 1991), prairie dogs (Muller *et al.*, 1987), ostriches (Frazier *et al.*, 1993), and horses (Diab *et al.*, 2013). *C. difficile* is a rod-shaped strict anaerobe. Cycloserine-cefoxitin-fructose agar (CCFA) is a commonly used selective medium for *C. difficile.* Cultures are incubated under anaerobic conditions at 35–37°C. When grown on blood agar, *C. difficile* colonies are nonhemolytic and gray, and have a slightly raised umbonate profile with filamentous edges and a ground-glass appearance. Colonies grown on blood agar have fluorescence under ultraviolet light. *C. difficile* forms acid from glucose and fructose, but is negative on lactose, maltose, and sucrose. Two closely related exotoxins, Toxin A and Toxin B, are produced by *C. difficile.* Recent taxonomic classification support placement of *C. difficile* and its close relatives within the family Peptostreptococcaceae. The authors suggested renaming it *Peptoclostridium difficile* (Yutin and Galperin, 2013).

Epizootiology It is estimated that *C. difficile* spores germinate and establish infection less than 10 h after ingestion. Spores rapidly transit through the upper gastrointestinal tract and colonize the colon and cecum. Spore shedding begins less than 2 h postingestion. When C57BL mice were challenged with 10^8 CFU of *C. difficile* spores, severe CDI signs developed and all mice were clinically affected by 48 h postchallenge (Chen *et al.*, 2008).

Specific methods to control and prevent *C. difficile* infections in mice have not been described. Given the method of transmission of *C. difficile* and *C. perfringens* are via ingestion or spores, these clostridia can probably be excluded from mouse colonies by maintaining strict husbandry practices, robust sanitation, and use of autoclaved feed, bedding, cages, and cage accessories. Sudden dietary changes should be avoided and antibiotics should be used judiciously to minimize disruption of the normal gut microbiota of mice.

Diagnosis of *C. difficile*-associated disease is generally based on detection of cytotoxin using a tissue culture cytotoxicity assay. PCR assays for detection of both *C. difficile* and its cytotoxins have been developed (Eastwood *et al.*, 2009). There are no published regimens specifically for the treatment of natural *C. difficile* infections in mice. Oral doses given twice daily of 2 mg vancomycin for 7 days to experimentally infected gnotobiotic mice caused a 2- to 3-log decrease in vegetative bacterial cell count and no detectable cytotoxin. Bacterial counts and cytotoxin levels returned to previous levels after treatment was discontinued.

Clinical Signs Untreated mice are relatively resistant to infection with *C. difficile* and do not develop fatal infections, although these mice can become asymptomatic carriers that persistently shed low numbers of spores (Lawley *et al.*, 2009). Susceptibility of mice to infection must be induced by disrupting the microbiota through antibiotic treatment. Brief exposure to environmental

spore contamination is sufficient for transmission of *C. difficile* to naïve but susceptible mice. The CDI transmission model has been used to demonstrate that clindamycin treatment of asymptomatic carriers of *C. difficile* can inadvertently trigger the excretion of high levels of spores (Lawley *et al.*, 2009). A C57BL mouse model of recurrence/relapse CDI has been reported (Sun *et al.*, 2011). The primary bout of CDI induced little or no protective antibody response against *C. difficile* toxins and mice continued shedding *C. difficile* spores. Antibiotic treatment of surviving mice induced a second episode of diarrhea. A simultaneous reexposure of mice to *C. difficile* bacteria or spores elicited a full clinical spectrum of CDI similar to that of the primary infection. Immunosuppressive agents resulted in more severe and fulminant recurrent disease. Vancomycin treatment only delayed disease recurrence; however, neutralizing polysera against both TcdA and TcdB completely protected mice against CDI relapse (Sun *et al.*, 2011). A recent study in C57BL mice demonstrated that antibiotic-mediated alteration of the gut microbiome favors a global metabolic profile, and therefore increases susceptibility to *C. difficile* clinical diseases (Theriot *et al.*, 2014).

C. difficile is not tissue invasive and only toxigenic strains are associated with disease. Experimental *C. difficile* infections include diarrhea, cecitis, polymorphonuclear cell infiltration of the lamina propria, inflammation, pseudomembrane formation, and death.

Differential Diagnosis *C. difficile*-induced diarrhea is most often associated with antibiotic treatment. Other clostridial diseases in mice must be ruled out as well as other enteric pathogens in mice causing diarrhea and mortality. *Salmonella* spp. and *C. rodentium* should be considered in the differential diagnosis.

CLOSTRIDIUM PERFRINGENS

Etiology *Clostridium perfringens* is associated with a number of diseases in domestic animals and humans. *C. perfringens* is a nonmotile, rod-shaped, encapsulated, anaerobic bacterium measuring 4–8 µm in length and 0.8–1.5 µm in diameter (Murray *et al.*, 2002). *C. perfringens* grow rapidly on blood agar, and colonies are smooth, round, and grayish in color, and are surrounded by a double zone of hemolysis. *C. perfringens* is grouped into five types based on the production and secretion of four major toxins. *C. perfringens* produces a number of other virulence-enhancing toxins and hydrolytic enzymes. The most significant of these is probably enterotoxin, released with the bacterial spore after cell lysis.

Epizootiology *C. perfringens* is most likely acquired by the ingestion of spores that originated in the soil or in the intestinal tract of a carrier animal. The organism can be a member of the normal microbiota in human and domestic animals. Factors that have been associated with the proliferation of the organism of these species include poor husbandry and sudden dietary changes (Quinn *et al.*, 2002). Methods to control and prevent *C. perfringens* infections have not been evaluated in mice. Because the bacterium is most likely acquired by the ingestion of spores, it can probably be excluded from mouse colonies by maintaining good sanitation and sterilizing feed, bedding, cages, and cage accessories. Sudden dietary changes have also been associated with proliferation of the organism and should be avoided if possible (Quinn *et al.*, 2002).

Clinical Signs Only a few reports in the literature exist describing clinical disease associated with *C. perfringens* infection in mice (Matsushita and Matsumoto, 1986; Rozengurt and Sanchez). Disease has been observed in mice of both sexes, from 2 to 32 days old, and in female mice of breeding age. Clinical signs have included hunched posture, ruffled hair coat, enlarged painful abdomen, soft or impacted feces, hindquarter paralysis, and dyspnea. Sudden death without premonitory signs has also been reported. The toxin types of *C. perfringens* isolated from these cases were reported to be non-type A (Matsushita and Matsumoto, 1986), type B (Rozengurt and Sanchez, 1999), and type D (Clapp and Graham, 1970). Mucosal necrosis in both the large and small intestine is a consistent finding on microscopic examination of tissues from mice with clinically apparent *C. perfringens* infections.

Differential Diagnosis *C. perfringens* produces a number of major and minor toxins. Different types of the bacterium produce different toxins which account for different disease outcomes. *C. perfringens* type A is a constituent of the normal microbiota of the intestine of humans and other animal species. Bacterial culture should be obtained from live or recently dead animals, and placed in anaerobic transfer medium for transport to a microbiology laboratory and should be cultured soon after their arrival. A presumptive diagnosis for *C. perfringens* can be based on the presence of large gram-positive rods in fecal smears or in histologic sections of intestines (Quinn *et al.*, 2002). Definitive diagnosis is based on toxin identification.

Mice treated with chlortetracycline hydrochloride in drinking water at a level of 11 mg/l for 2 weeks have eliminated *C. perfringens*-associated disease (Matsushita and Matsumoto, 1986). Penicillin G in the diet or changing the diet has also been reported to be effective in disease remission. *C. perfringens* treatments in domestic species include ampicillin, amoxicillin-clavulanate, tylosin, clindamycin, metronidazole, and bacitracin (Marks, 2013; McGorum *et al.*, 1998). Commercially available bacterins for use in mice were not effective in controlling the disease (Clapp and Graham, 1970).

Research Complications Clostridia are large, rod-shaped, gram-positive anaerobic bacteria. Naturally occurring clostridial infection in mice is rare. Epizootics of *C. 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. *C. perfringens* also has been isolated from sporadic cases of necrotizing enteritis in recently weaned mice.

CLOSTRIDIUM PILIFORME – *TYZZER'S DISEASE* (*Fujiwara and Ganaway, 1994; Ganaway, 1982; Ganaway* et al., *1971; Percy and Barthold, 2007*)

Etiology Tyzzer's disease is named for Ernest Tyzzer, who first described it in a colony of Japanese Waltzing mice. The causative organism, *C. piliforme* (formerly *Bacillus piliformis*), is a long, thin, gram-negative spore-forming bacterium that appears to require living cells for *in vitro* growth. It has not been grown successfully on cell-free media, but it can be propagated by inoculation of susceptible vertebrates, in select cell lines, the yolk sac of embryonated eggs, or hepatocyte cell cultures obtained 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 varies. 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, and young age, may predispose mice to Tyzzer's disease. Susceptibility and resistance also are influenced by host genotype. It has been shown, e.g., 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. The role of T cells in resistance is not clear, because susceptibility among athymic mice appears to vary (Livingston *et al.*, 1996). However, the involvement of T cells can be inferred by the fact that several interleukins modulate resistance and susceptibility. Depletion of neutrophils or NK 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. 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. Although the vegetative form of *C. piliforme* is unstable, 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 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.

Diagnosis Tyzzer's disease is diagnosed most directly by the demonstration of characteristic intracellular organisms in tissue sections of liver and intestine. Bundles of long, slender rods occur in the cytoplasm of viable cells bordering necrotic foci, especially in the liver (Fig. 3.46) 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, Giemsa stains, or periodic acid-Schiff stains are usually required for visualization of the organism. PCR and serologic assays are readily available at diagnostic laboratories. Older supplemental procedures included inoculation of cortisonized mice or embryonated eggs

FIGURE 3.46 *C. piliforme* in the liver on a mouse with Tyzzer's disease (Warthin–Starry stain).

with suspect material, followed by histological or immunocytochemical demonstration of organisms in tissues.

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 *C. 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).

p. Mycobacteriosis

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

Epizootiology Mycobacteria are widespread in water and soil. Their presence in laboratory mice would indicate a significant break in husbandry practices. 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. *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.

Clinical Signs *M. avium-intracellulare* infection is typically subclinical but mice have developed granulomatous pneumonia (Waggie *et al.*, 1983).

Pathology Lesions are classically a chronic granulomatous disease with granulomas, Langhans giant cells, and concurrent presence of acid-fast bacteria in various organs including the lungs, liver, spleen and lymph nodes. *M. lepraemurium* may cause 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 the lung, skeletal muscle, myocardium, kidneys, nerves, and adrenal glands. The histologic hallmark is perivascular granulomatosis with accumulation of large, foamy epitheloid macrophages (lepra cells) packed with acid-fast bacilli.

Diagnosis Acid-fast bacilli in lesions are the hallmark of presumptive diagnosis of mycobacteriosis. Definitive diagnosis results from positive culture which takes days to weeks to rule out or positive PCR assays which are more time-efficient but require associated expertise.

Differential Diagnosis Other bacterial species that cause granulomatous lesions in mice.

Research Complications Natural infection is very rare.

q. Proteus Infection

Etiology *Proteus mirabilis* is a ubiquitous gram-negative organism that can remain latent in the respiratory and intestinal tracts of normal mice (Percy and Barthold, 2007).

Epizootiology *Proteus mirabilis* colonizes the intestinal tract of most humans and is commonly found in research mice unless specifically excluded.

Clinical Signs Clinical disease can occur following stress or induced immunosuppression. Immunodeficient mice have a heightened susceptibility to pathogenic infection.

Pathology *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.

Diagnosis Culture recovery of *Proteus mirabilis* as a predominant or single isolate confirms an opportunistic local or systemic infection.

Differential Diagnosis Gram-negative bacterial infections.

Research Complications Natural infections are typically isolated cases.

r. Leptospirosis (Percy and Barthold, 2007)

Etiology Leptospirosis remains one of the most common zoonoses transmissible from rodents (Desvars *et al.*, 2011) but is exceedingly rare in laboratory mice. Infection with *Leptospira interrogans* serovar *ballum* has been reported on several occasions (see Chapter 29).

Epizootiology *Leptospira* are gram-negative organisms that, after a septicemic phase, establish persistent infection in the renal tubules and are periodically excreted in the urine.

Clinical Signs Natural infection is subclinical and causes no significant lesions. Experimental infections can result in severe vascular, hepatic and renal lesions dependent on serovar, mouse strain and immunocompetency.

Diagnosis 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. PCR assays are reliable for preliminary diagnosis.

Differential Diagnosis Not applicable in research colonies.

Research Complications Persistent murine infections associated with active shedding present a zoonotic hazard for humans; therefore, infected mice should be culled. Elimination of infection from highly valuable mice requires rederivation.

4. Chlamydial Diseases (Percy and Barthold, 2007)

a. Chlamydia Infection

Etiology *Chlamydia trachomatis* is an intracellular organism that produces glycogen-positive intracytoplasmic inclusions (elementary bodies). *C. trachomatis* causes ocular and urogenital disease in humans. However, at least one strain historically referred to as the 'Nigg agent' after Clara Nigg, is most recently classified as *Chlamydia muridarum* and is used experimentally to model human chlamydia infection.

Epizootiology Mice are susceptible to natural infection and experimental infection with *C. trachomatis* and *Chlamydophila psittaci*, especially immunodeficient mouse strains.

Clinical Signs Natural infections are typically subclinical but persistent.

Pathology *C. muridarum* is also known as the 'mouse pneumonitis agent' due to severe acute infection which is characterized by ruffled fur, hunched posture, and labored respiration due to interstitial pneumonitis and death in 24 h. Mice with more chronic infections may develop progressive emaciation and cyanosis of the ears and tail. Experimental infections to model human venereal chlamydia infections will develop hydrosalpinx, cervical, and vaginal infections in female mice and urethritis in male mice.

Diagnosis *Chlamydia* can be diagnosed by impression smears stained with Giemsa or Macchiavello stains, cell culture, or inoculation of embryonated eggs. PCR and sequencing can be used to speciate the type of chlamydia.

Differential Diagnosis *C. muridarum*, *C. trachomatis*, and *C. psittaci* are included in the differential diagnosis.

Research Complications *Chlamydia* is a rare spontaneous infection in research mice; its potential significance is low.

5. Mycotic Diseases

a. Pneumocystosis

Etiology *Pneumocystis murina* (*Pm*) is a common opportunistic organism of laboratory mice and other mammals. When first described by Chagas in 1909, *P. carinii* was misidentified as *Trypanosoma cruzi* and was considered a protozoan (Chagas, 1909). It was renamed as a new species, *P. carinii*, when observed in a rat in 1912 (Delanoë, P. and Delanoë, M. 1912). *P. carinii*, however, has now been grouped taxonomically with the fungi based on DNA analysis and the homology of *P. murina* housekeeping genes with those found in fungi (Edman *et al.*, 1989; Stringer *et al.*, 2002; Wakefield *et al.*, 1992). These DNA studies and apparent differences of host susceptibility prompted a new name, *P. jiroveci*, for pneumocystis isolated from humans (Stringer *et al.*, 2002; Frenkel, 1999). *P. carinii* is now used to name the organism in rats and *P. murina*, the organism in mice.

Clinical Signs *Pm* infection is subclinical 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). B cells have also been shown to be critical to clearance of infection and the mechanism appears only partially related to IgG and has a more important role in promoting activation and expansion of T cells (Lund *et al.*, 2003). B cells may also protect early hematopoietic progenitor activity during systemic responses to pneumocystis infection (Hoyt *et al.*, 2014). 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 *Pm* is known to infect a number of mammalian hosts, including ferrets, rats, mice, and humans. *Pm* 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, infection is assumed to be present in mouse colonies unless ruled out by extensive surveillance. Although these organisms appear morphologically similar, there are antigenic and genetic differences among *P. murina* isolated from different hosts (Weinberg and Durant, 1994; Cushion, 1998). Furthermore, studies indicate that *P. carinii* isolated from one host species is unable to survive and replicate after inoculation into a different immunodeficient host species (Gigliotti *et al.*, 1993b).

Pm infection also occurs in human beings, but transmission between rodents and human beings has not been documented. *Pm* is transmitted by aerosol and establishes persistent, quiescent infection in the lungs of immunocompetent mice. Prenatal infection has not been demonstrated.

Pathology *Pm* 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. 3.47). Histopathological changes are characterized by interstitial alveolitis with thickening of alveolar septa from proteinaceous exudate and infiltration with mononuclear cells (Fig. 3.48) (Roths *et al.*, 1990). Alveolar spaces may contain vacuolated eosinophilic material and macrophages. Special stains are required to visualize *Pm.* Silver-based stains reveal round or partially flattened 3- to 5-mm cysts in affected parenchyma (Fig. 3.49). In florid cases, alveolar spaces may be filled with cysts, but cysts may be sparse in mild cases. Disease can be especially severe when subclinically infected immunodeficient mice are reconstituted with competent immune cells that subsequently promote pneumonitis.

Diagnosis Respiratory distress in immunodeficient mice should elicit consideration of pneumocystosis. Pathologic examination of the lung, including silver

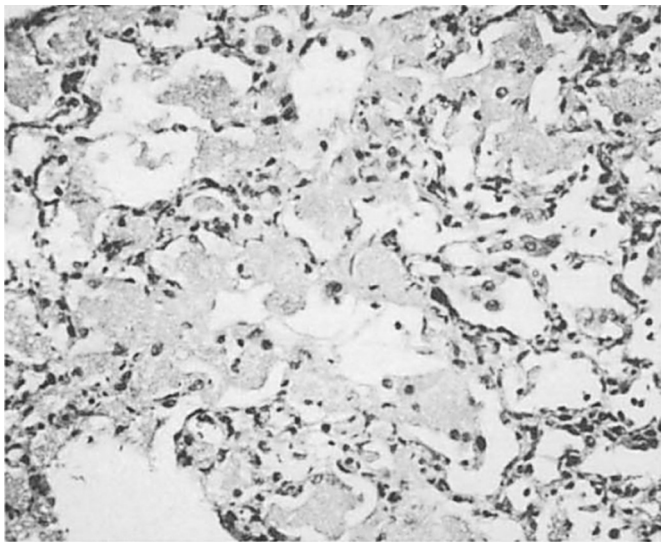

FIGURE 3.48 *Pneumocystis* pneumonia, illustrating hypercellular alveolar septa and alveoli containing proteinaceous exudate and macrophages.

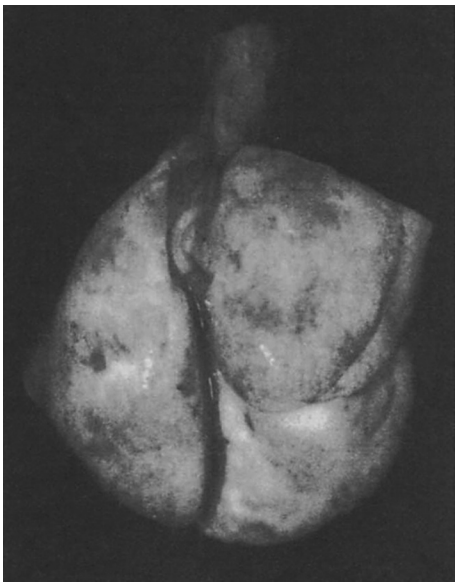

FIGURE 3.47 Lung from a mouse with *Pneumocystis* pneumonia that has failed to collapse after removal.

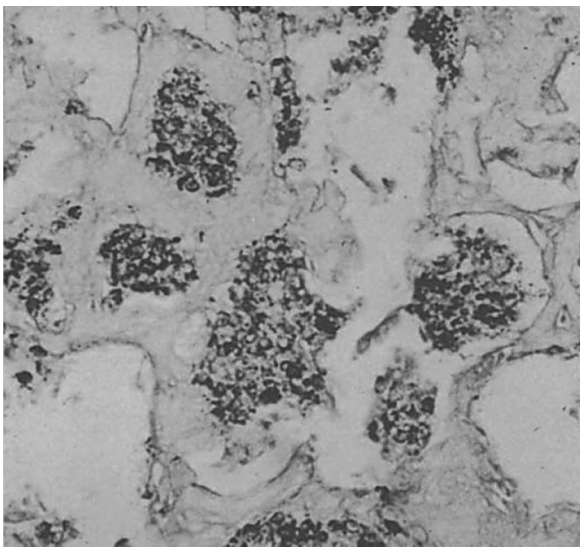

FIGURE 3.49 *Pneumocystis* pneumonia, illustrating *Pneumocystis* cysts in alveoli (Gomori methenamine–silver stain).

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

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

b. Dermatomycosis (Ringworm) (Godfrey, 2007)

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. *T. 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 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.

6. Parasitic Diseases

a. Protozoal Diseases (Wasson, 2007)

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 subclinical, 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 the 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, e.g., 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.

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 *S. muris* infection is usually subclinical 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 these 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 occasional 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.

Tritrichomoniasis *T. 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).

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 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 subclinical. 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.

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.

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.

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. *E. 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, 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. PCR-based assays may also be useful.

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, histopathology, and PCR. 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 (Pritchett, 2007; Baker, 2007)

HYMENOLEPIS *(RODENTOLEPIS) NANA* *(DWARF TAPEWORM) INFESTATION*

Etiology *Hymenolepis (Rodentolepis) nana*, the dwarf tapeworm, infects mice, rats, and humans although the zoonotic risk has been questioned (Macnish *et al.*, 2002). Adults are extremely small (25–40 mm) and have eggs with prominent polar filaments and rostellar hooks (Fig. 3.50).

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 (*R. 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.

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. *R. nana* can be differentiated from another species of rodent tapeworm, *H. diminuta*, by the fact that *R. 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 an 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 *R. nana* can directly infect humans, proper precautions should be taken to avoid oral contamination during handling of rodents (see Chapter 28).

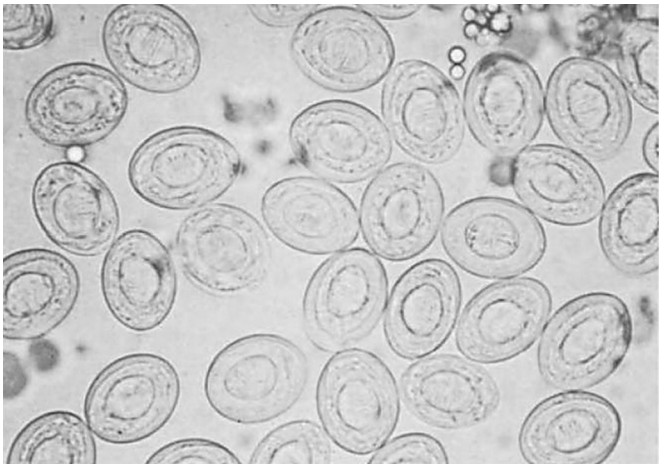

FIGURE 3.50 Eggs of *Hymenolepis (Rodentolepsis) nana*.

Hymenolepis microstoma is found in the bile ducts of rodents and could be confused with *R. nana* in the mouse. However, the location of the adult as well as the large size of *H. microstoma* eggs compared with those of *R. 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)

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 (1.1–1.5 mm). Eggs are flattened on one side and have pointed ends (Fig. 3.51). The nucleus fills the shell and is frequently at a larval stage when eggs are laid.

Clinical Signs Infestation is usually subclinical, 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 national survey revealed that more than 30% of barrier colonies and about 70% of conventional colonies were affected (Jacoby and Lindsey, 1997; Carty, 2008). *Syphacia obvelata* infestation can occur unexpectedly in commercial barrier murine colonies, resulting in widespread dissemination of the parasite into academic mouse colonies. 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 h. 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 *Aspiculuris tetraptera*. Because the life cycle of *Syphacia* is much shorter than that of *Aspiculuris*, 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).

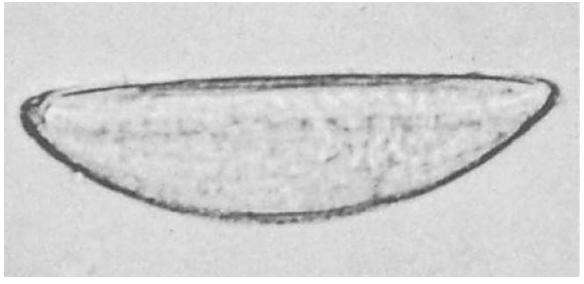

FIGURE 3.51 *Syphacia obvelata* egg.

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. *Aspiculuris 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). PCR assays are increasingly being used to augment traditional diagnostic methods and to discriminate between pinworm species (Dole *et al.*, 2011). PCR panels for pinworm detection using fecal pellets are available from commercial diagnostic laboratories.

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). Fenbendazole diets can be fed with 1 week on/1 week off rotation with normal chow although the potential impact on experimental data must be considered (Duan *et al.*, 2012; Gadad *et al.*, 2010; Landin *et al.*, 2009). Prevention of reinfestation requires strict isolation because *Syphacia* eggs become infective as soon as 6 h 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.

ASPICULURIS TETRAPTERA *(MOUSE PINWORM) INFECTION*

Etiology *Aspiculuris tetraptera* is the other major oxyurid of the mouse and may coinfect mice carrying *S. obvelata.* Females are 2.6–4.7 mm long, and males are slightly smaller. The eggs are ellipsoidal (Fig. 3.52).

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*, infestations appear in somewhat older mice; heaviest infestation is expected in 5–6 weeks after initial exposure. Infection is usually subclinical, 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).

Diagnosis *Aspiculuris 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.

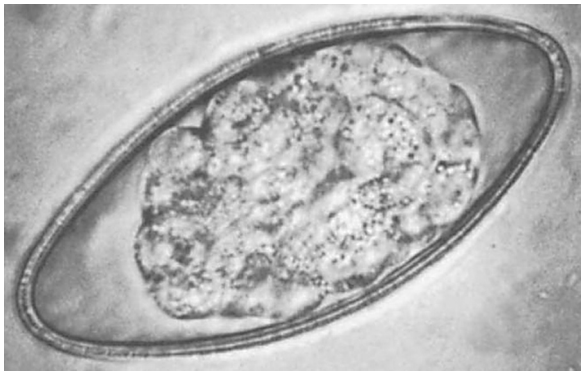

FIGURE 3.52 *Aspiculuris tetraptera* egg.

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).

d. Acariasis (Mite Infestation) (Baker, 2007)

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 species are listed in Table 3.14. 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

TABLE 3.14 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*	

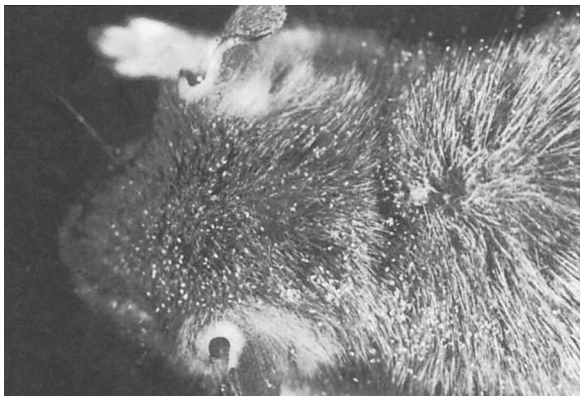

FIGURE 3.53 *Acariasis.*

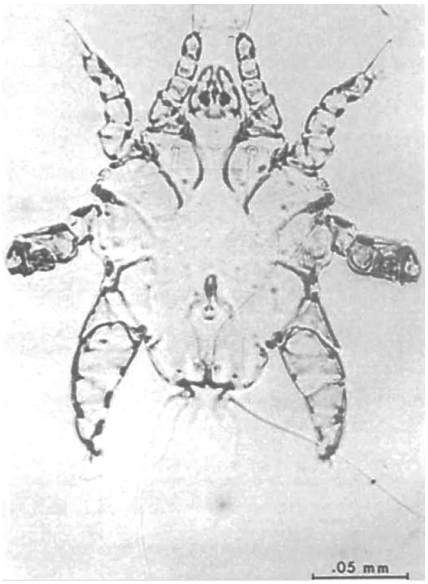

FIGURE 3.54 *M. musculinus* male. *From Weisbroth (1982).*

the head, neck, and withers (areas least amenable to grooming), but in severe cases, all areas of skin can be infested (Fig. 3.53). 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 (Jacoby and Lindsey, 1997; Carty, 2008) reported mite infestations in 40% of colonies. Acarids spend their entire lives on the host. Populations 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, e.g., 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 in the dermis.

Diagnosis Classic methods of detection include direct observation of the hair and skin of dead or anesthetized mice. 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.

Detecting mites in sentinels exposed to dirty bedding from colony animals has been reported to be unreliable (Lindstrom *et al.*, 2011). Thus, PCR assays offered by commercial diagnostic laboratories are increasingly being used to augment traditional diagnostic methods and to test individual animals or equipment using a swabbing technique; samples can be pooled to decrease cost (Jensen *et al.*, 2013).

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. 3.54). *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. 3.55), whereas *Radfordia* has two claws of unequal size on the terminal tarsal structure of its second pair of legs (Fig. 3.56). 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 Ivermectin can be used topically, in drinking water or as a medicated feed and often is the first-choice approach for attempting eradication although cost and potential toxicity are concerns. Because of potential differences in

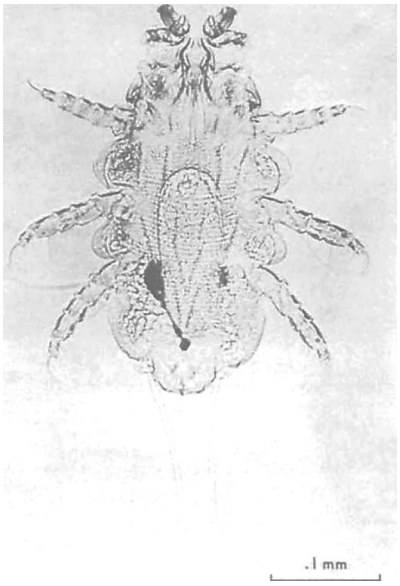

FIGURE 3.55 *M. musculi* female. *From Weisbroth (1982).*

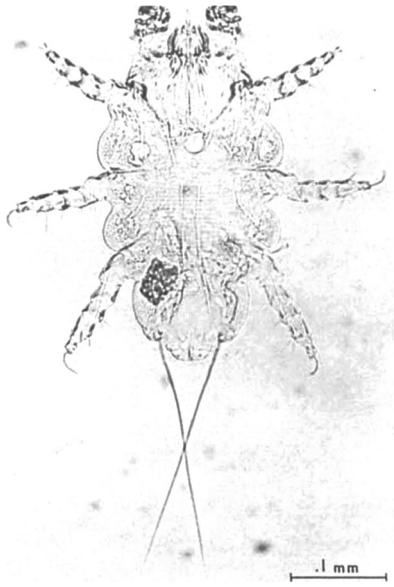

FIGURE 3.56 *R. affinis* female. *From Weisbroth (1982).*

blood–brain barrier permeability to ivermectin, pilot treatments should be evaluated. For large facilities, ivermectin medicated feed may be an attractive option (Ricart Arbona *et al.*, 2010). For valuable lines of mice, rederivation may be cost- and time-effective. Control and prevention programs should be carried out on a colony-wide 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 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 displace *Myobia* during heavy infestations. It has some predilection for the 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 stages, 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 h.

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 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 cyst-like 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 exam.

Mesostigmoid Mites Rarely, blood-sucking *Ornithonyssus bacoti* and *Laelaps echidnina*, normally limited to wild rodents, can also infect laboratory rodent colonies (Watson, 2008; Fox, 1982). These mites may also transiently bite humans and can transmit zoonotic infections (see Chapter 29). Unlike the more common rodent fur mites, mesostigmoid mites live off the host and can travel a long distance in search of a blood meal. They access research colonies via contaminated supplies or wild rats and mice gaining access to the facility.

e. Pediculosis (LICE) (Percy and Barthold, 2007)

Polyplax serrata, the mouse louse, is encountered in wild mice but no longer is a significant issue in research colonies. Eggs are deposited at the base of hair shafts and nymph stages and adults can be found principally on the dorsum. *P. serrata* causes pruritus with associated dermatitis, anemia and debilitation and historically is the vector for *Mycoplasma coccoides*.

B. Metabolic and Nutritional Diseases

1. Amyloidosis

Amyloidosis is caused by the deposition of insoluble (polymerized), mis-folded amyloid protein fibrils in organs and/or tissues. 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 older C57BL mice. Other strains, such as BALB/c and C3H are highly resistant to amyloidosis (Percy and Barthold, 2007). 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. In reactive amyloid A (AA) amyloidosis, serum AA (SAA) protein forms deposits in mice, domestic and wild animals, and humans that experience chronic inflammation. AA amyloid fibrils are abnormal β-sheet-rich forms of the serum precursor SAA, with conformational changes that promote fibril formation. Similar to prion diseases, recent findings suggest that AA amyloidosis could be transmissible in mice and other species (Murakami *et al.*, 2014). Amyloid fibrils induce a seeding-nucleation process that may lead to development of AA amyloidosis. Amyloidosis can shorten the life span of mice and can be accelerated by stress from intercurrent disease.

Amyloid appears histologically 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. 3.57).

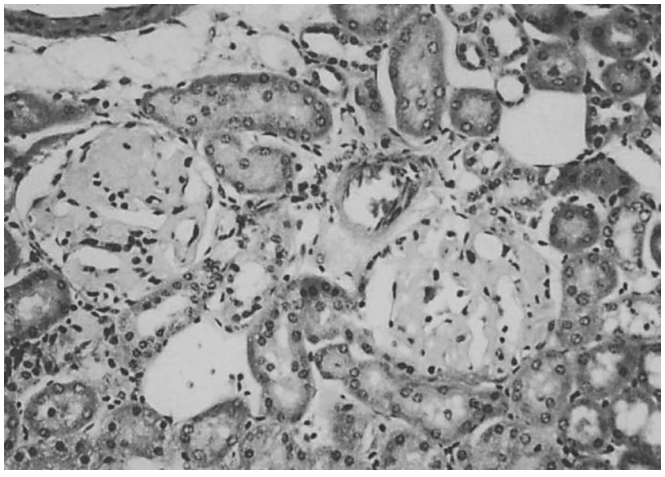

FIGURE 3.57 Renal amyloidosis with prominent amyloid deposition in glomeruli.

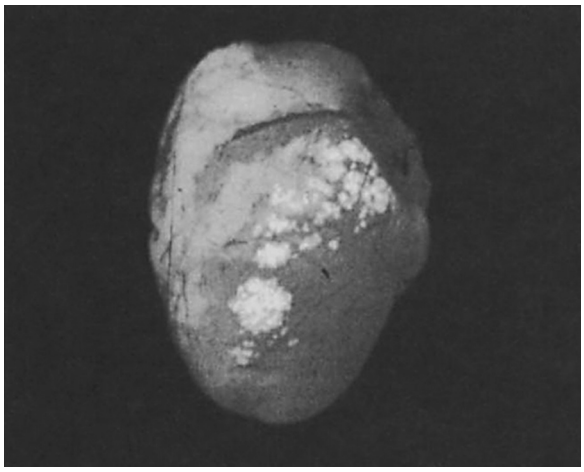

FIGURE 3.58 Epicardial mineralization.

2. Soft Tissue Mineralization

Naturally occurring mineralization of the myocardium and epicardium and other soft tissues is a common finding at necropsy in some inbred strains of mice. Although this condition is usually an incidental finding at necropsy, interference with organ function such as the heart cannot be ruled out if lesions are severe. 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. 3.58), 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. Ectopic mineralization is associated clinically with skin and vascular connective tissue conditions in humans and mouse models have been developed to study metastatic and dystrophic tissue mineralization (Li and Uitto, 2013). Pseudoxanthoma elasticum (PXE), a heritable ectopic mineralization disorder in humans, is caused by mutations in the ABCC6 gene. Knockout Abcc6$^{-/-}$ mice model the histopathologic and ultrastructural features of PXE, notably with mineralization of the vibrissae dermal sheath, serving as a biomarker of tissue mineralization (Benga *et al.*, 2014). Other inbred mouse strains, including KK/HlJ and 129S1/SvImJ, also develop vibrissae dermal mineralization and have an SNP (rs32756904) in the Abcc6 gene associated with low levels of ABCC6 protein expression in the liver. DBA/2J and C3H/HeJ mice have the same polymorphism and low ABCC6 protein levels; however, these mice only develop tissue mineralization when fed an experimental diet enriched in phosphate and low in magnesium.

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 h, 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 (Tobin et al., 2007)

Vitamin deficiencies in mice have not been thoroughly described. Unfortunately, much of the information that does exist reflects work carried out 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 A is recognized for its importance in development of the immune system (Ross, 2012) and knockout mouse models have been used to demonstrate genetic polymorphisms in humans that negatively regulate intestinal β-carotene absorption and conversion to retinoids in response to vitamin A requirements for growth and reproduction (von Lintig, 2012). Vitamin E deficiency can cause convulsions and heart failure, as well as muscular dystrophy and hyaline degeneration of muscles. Two knockout mouse models of severe vitamin E deficiency were independently developed and lack α-tocopherol transfer protein (α-TTP), a gene that controls plasma and tissue α-tocopherol concentrations by exporting α-tocopherol from the liver. *Ttpa*$^{-/-}$ mice have very low to undetectable levels of α-tocopherol and are infertile. The phenotype includes neuronal degeneration associated with progressive ataxia and age-related behavioral defects (Yu and Schellhorn, 2013). 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. The gulonolactone oxidase knockout mouse (*Gulo*$^{-/-}$) on the C57BL/6 background requires vitamin C supplementation although the plasma ascorbate concentration of *Gulo*$^{-/-}$ mice fed a vitamin C-deficient diet is maintained at 15% of wild-type concentrations, suggesting an uncharacterized pathway to generate a small amount of ascorbate (Yu and Schellhorn, 2013). The *Gulo*$^{-/-}$ mouse has become the model of choice in studying the role of vitamin C in complex diseases. Vitamin C production has been successfully restored in *Gulo*$^{-/-}$ mice using adenovirus vectors, making it possible to robustly manipulate physiological ascorbate concentrations in an inbred mouse.

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 arachidonic acid.

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

The significant syndrome of ulcerative dermatitis (UD) is a common idiopathic skin lesion that causes morbidity and early euthanasia losses in C57BL/6 and related lines of mice. Significant pruritus leads to skin trauma associated with opportunistic bacterial infection and deep dermal ulcerations. Initial signs include alopecia and papular dermatitis, which usually occur over the dorsal trunk (Fig. 3.59). Progressive inflammation can be halted, sometimes reversed, by nail trimming and therapy with a wide spectrum of topical or systemic antibiotics, steroids, and other drugs such as Vitamin E and aloe, all of which speak to the frustrating search for a primary etiology. Treatment should be based on microbiological culture and sensitivity and screening for ectoparasites as hypersensitivity to acariasis has been proposed. 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 dietary fat with mice on high fat or *ad libitum* diets being more susceptible than those on restricted diets (Neuhaus *et al.*, 2012).

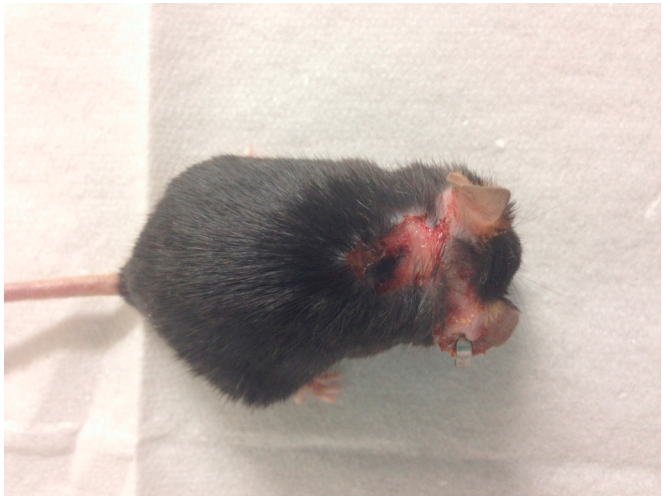

FIGURE 3.59 Dorsal chronic ulcerative dermatitis in a C57BL/6. *Courtesy of Abigail Powell.*

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). This disorder has been described as acute intestinal pseudo-obstruction (IPO) in C57BL/6 mice free of known pathogens (Feinstein *et al.*, 2008). Lactating mice are either found dead or becoming moribund. Segments of the small intestine become distended with fluid contents and histologically there is apoptosis of the villus epithelium of the small intestine and superficial epithelial cells of the large intestine. The enteric nervous system appears morphologically normal but necrotic enterocytes, mucosal erosions, and acute mucosal inflammation are commonly observed. There is no strong evidence for metabolic issues such as hypocalcemia or low blood glucose. The direct cause is unknown but death probably results from sepsis secondary to loss of barrier function reflected in apoptosis of the gut epithelium during peak lactation.

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.

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 automatic watering system valves or water bottles, resulting in spills (hypothermia) or obstructed sipper tubes (dehydration), are a significant cause of husbandry-related morbidity. Shipping mice between facilities, irrespective of distance, warrants institutional guidelines to minimize exposure to temperature extremes. Reheat coils should be designed to fail in the closed position to avoid overheating holding rooms.

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. 3.60). 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 result from acute or chronic keratitis, injury (unilateral) and developmental defects; the latter may occur in combination with inherited microphthalmia in C57 black mice (Koch and Gowen, 1939). There is some evidence that the buildup of ammonia in mouse cages may contribute to inflammatory keratitis, because it can be controlled by increasing the frequency of cage cleaning. Corneal opacities and anterior polar cataracts are a developmental defect in inbred C57 black mice (Pierro and Spiggle, 1967). Corneal opacity may be associated with keratolenticular adhesions involving a persistent epithelial stalk of the lens vesicle, which normally disappears around day 11 of gestation (Koch and Gowen, 1939).

4. Malocclusion

Typically noted in runted or cachectic mice soon after weaning, malocclusion of the open-rooted, continually growing incisor teeth is an inherited trait expressed as poorly aligned incisors, especially of the lower incisors causing osteomyelitis, soft tissue abscesses, or necrosis in the lips or oral cavity. The incidence of inherited malocclusion varies with mouse strain (Petznek *et al.*, 2002). Malocclusion in older mice may be the result of trauma or oral neoplasia. Overgrown molar teeth have been associated with trauma to developing tooth buds.

5. Skin Trauma

Skin lesions can be caused by fighting, tail biting, and overgrooming such as whisker chewing. Barbering of facial hair and whiskers in subordinate mice by a dominant cagemate is common and may be solved by removing the dominant, normally haired mouse.

Hair or whisker chewing (barbering) has long been interpreted to be a manifestation of social dominance. Apparent dominant animals retain whiskers, whereas cagemates have 'shaved faces' (Fig. 3.61). 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

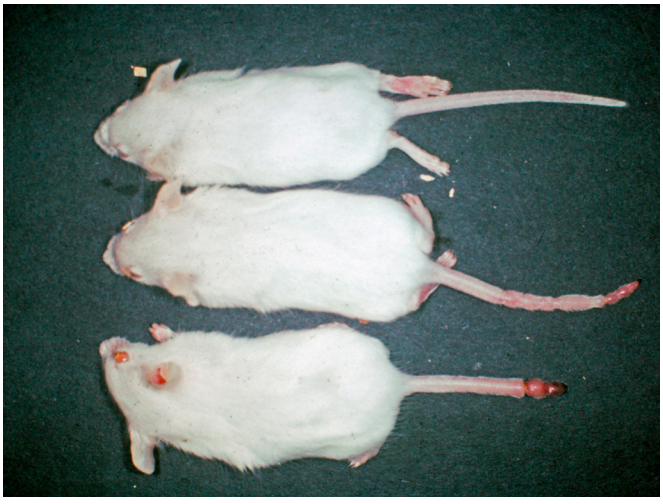

FIGURE 3.60 Ringtail, an idiopathic annular constriction around the tail of mice associated with low relative humidity.

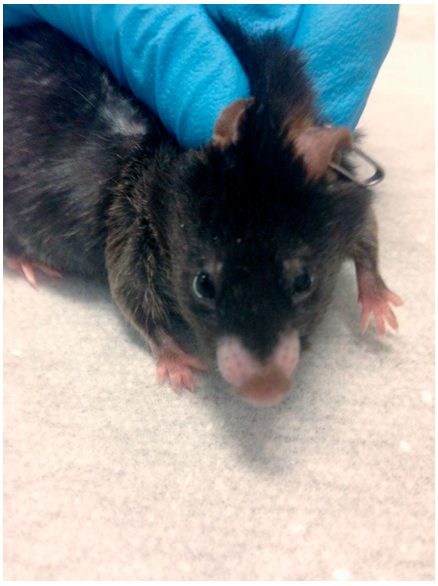

FIGURE 3.61 Barbering of the whiskers. *Courtesy of Abigail Powell.*

several weeks. Both sexes may engage in this activity, and sometimes females may be dominant. Barbering of whiskers and fur-plucking behavior in mice has been suggested to model human trichotillomania (compulsive hair plucking) because of similarities including elevated serotonin levels (Dufour *et al.*, 2010), 'barbers' predominately pluck hair from the scalp and around the eyes and the genitals; the behavior is female biased, and begins during puberty and is impacted by genetic background (Garner *et al.*, 2004).

Fighting is more common in male mice and more aggressive in some strains (SJL, FVB, BALB/c) with bite wounds typically located on the head, neck, shoulders, perineal area, and tail. Often one mouse in the cage is free of lesions and is the likely aggressor. Removal of the unaffected male may end the fighting or simply reorder the dominance order. Removing males for breeding and then regrouping them often results in fighting. For programs that produce sentinel mice in-house, castration is an option to reduce aggression in group-housed male sentinels (Lofgren *et al.*, 2012). Regional alopecia, especially around the muzzle, may result from abrasion against cage surfaces. Improperly diluted disinfectants may also cause regional hair loss. Ear tags used for identification may cause pruritis and self-induced trauma. Hair removal products or clipping prior to imaging or application of experimental compounds to the skin may cause pruritus 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, 2007)

Common idiopathic lesions in aging mice include cardiomyopathy (with or without mineralization or arteritis), chronic nephropathy (frequently with mineralization), myelofibrosis (fibrotic change in the bone marrow) especially in female mice, melanosis in the meninges, ovarian atrophy (with or without hyaline material), pigment (ceroid-lipofuscin), tubular or stromal hyperplasia, cystic endometrial hyperplasia, testicular tubular degeneration or mineralization, prostate atypical epithelial hyperplasia, gastric glandular epithelial hyperplasia, pancreatic islet cell hyperplasia, dental dysplasia of incisor teeth, pituitary hyperplasia of pars intermedia and pars distalis, cataracts, increased extramedullary hematopoiesis in spleen, and lymphocytic infiltrates or other inflammatory changes in various tissues, including Harderian gland, salivary gland, kidney, liver, gall bladder, nasal, trachea, thyroid, periovarian fat, epididymis, and urinary bladder. Lymphoma is also very common (Haines *et al.*, 2001).

1. Cardiovascular System

Spontaneous *atrial thrombosis* is rare in mice (<1% in 2-year-old mice) and appears to be strain-related, with a high prevalence in RFM mice. It also is more common in aged mice affected by kidney disease and amyloidosis. Organizing thrombi will be found usually in an enlarged, hyperemic left atrium and auricle and may be accompanied by amyloidosis. Affected mice may display signs of heart failure, particularly severe dyspnea. Induction of atrial thrombosis in B6C3F1 mice has been used to assess cardiovascular risk of chemical exposures (Yoshizawa *et al.*, 2005). *Myocardial and epicardial mineralization* is described above (Section III,B,2). Periarteritis, also known as arteritis, polyarteritis, or systemic arteritis, impacts older mice and lesions may be observed in multiple tissues, including the spleen, heart, tongue, uterus, testes, kidney, and urinary bladder. The media of the affected vessels is homogenous and intensely eosinophilic with hematoxylin and eosin stain. Fibrosis and mononuclear cells infiltrate the vessel wall. Experimental coronary arteritis with cardiac hypertrophy has been model in DBA/2 and other strains by intraperitoneal administration of mannoprotein–beta-glucan complex isolated from *C. albicans* (Nagi-Miura *et al.*, 2006).

2. Respiratory Tract

Hyperplasia of alveolar or bronchial epithelium occurs in old mice and must be differentiated from pulmonary tumors. Pulmonary histiocytosis, acidophilic macrophage pneumonia, and acidophilic crystalline pneumonia are synonymous morphologic descriptions of an idiopathic lung lesion that can be incidental or the cause of significant morbidity. Incidence varies with mouse strain or stock, with C57BL, 129S4/SvJae and Swiss mice and older mice in general particularly susceptible. Histologically, alveoli and bronchioles are filled with varying quantities of macrophages containing eosinophilic crystalline material (Ward *et al.*, 2001). The crystalline material consists of YM1 and/or YM2 chitanases and can be found in other tissues including the upper respiratory tract, stomach, gall bladder, and bone marrow where it is described as hyalinosis (Nio *et al.*, 2004).

3. Alimentary Tract

a. Stomach

Gastric lesions include crypt dilatation, submucosal fibrosis, adenomatous gastric hyperplasia, mineralization, and erosion or ulceration. Gastric ulcers have been induced by cold stress, food restriction (Rehm *et al.*, 1987), chemical injury (Yadav *et al.*, 2013), and gastritis and gastric tumors by helicobacter infection (Fox *et al.*, 2003). Germfree mice have reduced muscle tone in the intestinal tract. Cecal volvulus is a common cause of death in germfree mice and is caused by rotation of the large, thin-walled 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. Grossly observable black pigmentation in the spleen of C57BL/6 is normal and is melanosis caused by melanin deposition (Weissman, 1967). 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 importantly, fails to develop a thymus and thus has a severe deficit of T-cell-mediated immune function. Additionally, SCID mice, which lack both T and B lymphocytes, are used widely and are highly susceptible to opportunistic agents such as *Pneumocystis murina*. Specific immune deficits have become excellent models for studying the ontogeny and mechanisms of immune responsiveness (Table 3.12).

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, e.g., 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 proteinuria, 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 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. Preputial glands, especially of immunodeficient mice, can become infected with opportunistic or pathogenic bacteria. Spontaneous lesions in prostate, coagulating gland (anterior prostatic lobe), seminal vesicles, and ampullary glands were described in control B6C3F1 mice from 12 National Toxicology Program 2-year carcinogenicity and toxicity studies conducted in one of four different laboratories (Suwa *et al.*, 2002). Lymphocytic infiltration, inflammation, edema, epithelial hyperplasia, mucinous cyst, mucinous metaplasia, adenoma, adenocarcinoma, granular cell tumor, and glandular atrophy were variously observed in accessory sex glands.

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 ultimo-branchial 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 mellitus occurs in outbred swiss mice and genetic variants of several strains such as NOD mice (Lemke *et al.*, 2008).

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. A seizure syndrome in FVB mice has been described (Goelz *et al.*, 1998) and can be spontaneous or associated with tail tattooing, fur clipping, and fire alarms. Mice are most often female with a mean age of 5.8 months (range, 2–16 months) and can exhibit facial grimace, chewing automatism, ptyalism with matting of the fur of the ventral aspect of the neck and/or forelimbs, and clonic convulsions that may progress to tonic convulsions and death. Ischemic neuronal necrosis was consistently observed in these mice and is consistent with status epilepticus in humans.

10. Organs of Special Sense

a. Eye (Also See Corneal Opacities)

Unilateral and bilateral microphthalmia and anophthalmia are frequent (as high as 12%) developmental defects in inbred and congenic strains of C57BL mice, especially impacting the right eye and female mice. These conditions may first be recognized due to ocular infections, secondary to inadequate tear drainage. Other common findings include central corneal opacities, iridocorneal and corneal-lenticular adhesions, abnormal formation of the iris and ciliary body, cataracts, extrusion of lens cortical material with dispersion throughout the eye, failure of vitreous development, and retinal folding. These syndromes can be reproduced by exposure to alcohol at critical stages of embryogenesis when the optic cup and lens vesicle are developing and impacting normal development of other ocular structures, including the iris, ciliary body, vitreous, and retina (Smith *et al.*, 1994).

Retinal degeneration can occur as either an environmental or a genetic disorder (Chang *et al.*, 2007) 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. Mouse genetics have

been shown to be more important than potential light associated tissue injury (Serfilippi *et al.*, 2004a). Other strains such as C3H, CBA, and FVB are genetically predisposed to retinal degeneration because they carry 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). Presence of the *rd* gene in some mouse strains highlights that impaired vision must be a consideration when selecting strains for behavioral assays that rely on visual clues (Garcia *et al.*, 2004). 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 ears.

E. Neoplastic Diseases (Jones *et al.*, 1983–1993; Maronpot *et al.*, 1999; Percy and Barthold, 2007)

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 genome. 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 intrauterine infection or through saliva, sputum, urine, feces, or milk. The leukemia induced by a given endogenous virus is usually of a single histopathological type. Loss of function in nucleic acid-recognizing, TLR3, TLR7, and TLR9 can result in spontaneous retroviral viremia and acute T-cell lymphoblastic leukemia (Yu *et al.*, 2012). 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 lymphoblasts, 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 the 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 the 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 the 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. Erythroleukemia can be experimentally induced in mice by Friend spleen focus-forming virus (SFFV) which initially activates the erythropoietin (Epo) receptor and the receptor tyrosine kinase sf-Stk in erythroid cells, resulting in proliferation, differentiation, and survival. In a second stage, SFFV activates the myeloid transcription factor PU.1, blocking erythroid cell differentiation, and in conjunction with the loss of p53 tumor suppressor activity, results in the outgrowth of malignant cells (Cmarik and Ruscetti, 2010).

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, particularly in BALB/c mice. Mineral oil-induced plasmacytomas in BALB/c mice produce large amounts of endogenous retroelements such as ecotropic and polytropic murine leukemia virus and intracisternal A particles. Associated inflammation may promote retroelement insertion into cancer genes, thereby promoting tumors (Knittel *et al.*, 2014). Similar to other spontaneous cancers, plasmacytoma development in mice is inhibited by innate immune responses of NK cells which when activated by viruses will release γINF (Thirion *et al.*, 2014).

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.

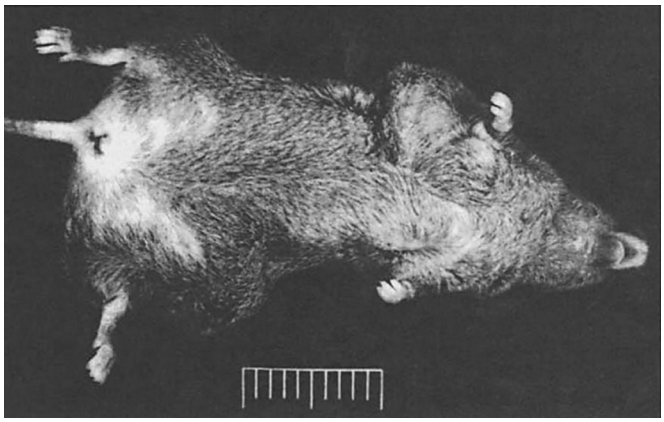

FIGURE 3.62 Mammary tumors in a C3H mouse.

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. 3.62). Histologically, mammary tumors have been categorized into three major groups: carcinomas, carcinomas with squamous cell differentiation, and carcinosarcomas. The carcinomas are divided into adenocarcinoma 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 horizontally transmitted virus such as MMTV-S can be determined 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. Mammary tumors are increased in frequency in C57BL $Apc^{+/-}$ female mice infected with *H. hepaticus* (Rao *et al.*, 2007).

3. *Liver*

Mice develop an assortment of liver changes as they age, including proliferative lesions which can progress 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 and *H. hepaticus* infections in susceptible strains of mice such as the A/JCr male mouse (Fox *et al.*, 1994; Ward *et al.*, 1994). The prevalence of spontaneous liver tumors in $B6C3F_1$ hybrids is increased by feeding choline-deficient diets or when infected with *H. hepaticus* (Hailey *et al.*, 1998). Tumors also can develop in mice exposed to environmental chemicals, many of which are carcinogenic or potentially carcinogenic (Hoenerhoff *et al.*, 2009).

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 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*

Given the rapid development of mouse strains genetically predisposed to neoplasia, the Mouse Tumor Biology Database maintained by Jackson Laboratory is a valuable centralized resource for the most current tumor descriptions. The database contains information on more than 4400 strains and substrains, 348 tissues and organs, over 42,000 tumor frequency records, and nearly 4800 histopathological images and descriptions.

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