The series of papers in this issue reflects the present state of knowledge on opportunistic infections of laboratory rodents and provides speculation into where the field is headed. What has not been covered is the phenomenal growth in knowledge of murine infectious diseases over the last 50 yr. Remarkably, more than 60 genera of overtly or opportunistically pathogenic viruses, bacteria, fungi, protozoa, helminths, and arthropods are known to infect laboratory mice (Percy and Barthold 1993). Wild mice and rats are host to an even longer list of agents that can potentially reenter domestic rodent colonies.

It is equally remarkable that although laboratory rodent populations are often free of these agents, many of these pathogens are still around. Coronaviruses (mouse hepatitis viruses in the mouse and sialodacryoadenitis viruses in the rat) are regularly monitored agents, but their sheer contiguity and mutability allow them continued and repeated access to rodent colonies. Pinworms, whose ova are resistant to desiccation and tend to drift in the air, are often the first pathogens to gain access to well-maintained barriers and the last to be eliminated (NRC 1991). Even rarely encountered agents continue to reappear in rodent populations. Polyoma virus of mice has been readily eliminated by simple husbandry practices for years (Barthold 1985), but it remains a virus of scientific interest that has been iatrogenically introduced into mouse colonies and has produced disease in immunodeficient mice (Sebesteny and others 1980). Ectromelia virus in mice has recently been incriminated as a contaminant of commercially available mouse serum, resulting in an outbreak of disease (Dick and others 1996). Freezers, transplantable tumors and biological products of murine origin continue to harbor murine pathogens. Entry of feral or wild rodents into laboratory rodent colonies is a constant threat. A case in point was infection of a “pathogen-free” mouse colony with lymphocytic choriomeningitis virus resulting from entry of wild mice into the animal room (Skinner and others 1977).

Furthermore, there are diseases and lesions in both mice and rats that have yet to be ascribed an etiology. As rodents become more microbiologically pristine, agents of lesser pathogenicity become recognized. In recent years, this has been the case for the discovery of novel parvoviruses. In the case of the mouse and rat parvoviruses, their discovery was heralded by development of new serological assays that used infected cells as an antigen substrate for immunofluorescence assays, rather than virion-specific assays such as hemagglutination inhibition, which utilizes purified virions as the substrate. The change in assays resulted in recognition of seroconversion of mice and rats to parvoviruses that shared conserved, cross-reactive nonstructural antigens with the known parvoviruses (minute virus of mice, rat virus, and Toolan H-1 virus). This discovery led to the recognition that these (originally termed “orphan”) parvoviruses were widespread among colonies of rodents in which the previously known minute virus of mice, rat virus, and Toolan H-1 had been eliminated. Retrospective serosurveys indicated that mouse parvovirus (MPV1) was widespread in mouse colonies for more than 20 yr before it was known to exist (Jacoby and others 1996). This scenario is reminiscent of events that took place in the 1970s, when complement fixation assays for mouse hepatitis virus antibody were replaced by more sensitive indirect fluorescent antibody and enzyme-linked immunosorbent assay methods (Smith 1983; Smith and Winograd 1986).

In addition, as genetic manipulation of mice increases, unexpected phenotypes, including overt or subtle immunomodulation, result in disease from opportunistic pathogens. Once a new entity is recognized, it often leads to a spurt of discovery of related agents or increased recognition of the agent. Once awareness of Helicobacter in mice was raised, it led to the recognition of several species of Helicobacter in mice (Fox and Lee 1997; Shen and others 1997) and Helicobacter infections in rats and hamsters (Franklin and others 1996; Haines and others 1998). The recognition of new parvoviruses in mice and rats led to the discovery of a related parvovirus in hamsters (Besselsen and others 1996). Similar events occurred with murine mycoplasmas, coronaviruses, and other agents of rodents.

The age of discovery for rodent infectious diseases is far from over. For example, rats free of all known pathogens develop pulmonary lesions that are suspected to have an infectious origin (Riley and others 1997). Several years ago, an uncharacterized polyoma-like virus was documented in athymic (nu) rats (Ward and others 1984), which recently
reappeared (unpublished). The prevalence and significance of this uncharacterized virus in immunocompetent rats has never been explored. Chronic respiratory disease of rodents has evolved as a disease syndrome that was originally considered to be multifactorial to one considered to be principally associated with *Mycoplasma* infection (Cassell and others 1979). As environmental, viral, and mycoplasmal factors were peeled away, cilia-associated respiratory bacillus was discovered (Ganaway and others 1985).

Finally, the world is full of emerging and reemerging pathogens that may, under opportune circumstances, become murine pathogens. The author is aware of enterovirus disease in mice associated with *Escherichia coli*, outbreaks of apparently infectious pancreatitis in rats, and an infectious enteritis in rats caused by a filterable agent (personal observations). Protozoan parasites with broad host range, such as *Cryptosporidia*, are becoming increasingly important contaminants of water and food supplies, which could very well lead to introduction to rodent colonies as anthropozoonotic diseases. Indeed, enteritis in young rats has been associated with *Cryptosporidium* (Moody and others 1991). Enteric diarrhea of infant rats ("IDIR") was recognized several years ago as an atypical rotavirus of probable human origin (Vonderfecht and others 1984). Will there be others? Absolutely.

**ISSUES THAT AFFECT MICROBIOLOGICAL MONITORING**

The article by Weisbroth and others (1998) raises the question of what is a pathogen, and how do you distinguish a primary pathogen from an opportunistic pathogen or a commensal? With the high sophistication of laboratory rodents, coupled with their increasingly immunocompromised status, these distinctions clearly blur. However, virtually none of the many murine pathogens is always pathogenic. Even the most overtly pathogenic agents like ectromelia virus is clinically silent when in the enzootic state or in genetically resistant adult mice (Bhatt and Jacoby 1987). Mouse hepatitis virus can cause epizootics with 100% mortality in infant mice, but the very same virus strain can become totally subclinical in subsequent litters of pups born to immune dams (Barthold and others 1982). MPV could be considered a trivial agent, since it induces no detectable gross or microscopic pathology in naturally or experimentally inoculated immunocompetent or immunodeficient mice. However, MPV has striking immunomodulatory effects because of its T-lymphocytotropism, even in adult mice (McKisic and others 1993). In the case of the laboratory mouse, subclinical effects of infection often have significant effects on physiological or immune responses.

There is no way to predict whether a particular infectious agent will affect research results, but there is no doubt that even the most subtle pathogens have that potential. Under select research circumstances, virtually all of the infectious agents that afflict mice and rats can and do alter research results. There will never be an absolute "list" of good and bad that *must* be, or *should* be, or *can* be excluded from rodent colonies. However, carte blanche serosurveys of every potential pathogen or opportunistic pathogen for colonies of rodents that do not require such scrutiny is a major and unnecessary cost factor in effective diagnostic programs. It is extremely important for laboratory animal professionals to become facile with the biology of laboratory rodents, infectious disease epidemiology, and the biological behavior of these varied agents to provide learned advice to research investigators about whether they can live with one or more of these agents, depending on his or her research program. The importance of "Risk Assessment," as discussed by White and others (1998), cannot be overemphasized.

Considering these issues, there remains a critical need to develop uniform standards for defining "pathogen free" and to standardize testing methods among institutions. This must be done to facilitate interinstitutional traffic of rodents, without jeopardizing recipient institutions or interfering with the progress of science. What lies ahead in the coming decade makes this a more pressing issue than ever.

**CURRENT TRENDS THAT WILL AFFECT THE FUTURE**

A number of forces will undoubtedly have an impact on the issue of rodent opportunistic infectious disease in the coming decade. These forces are discussed below and include the increasing (infectious disease) dichotomy between industry and academia, the profound changes in biomedical research animal use, opposing financial pressures, an attrition of the comparative medical academic infrastructure, and a renewed urgency for a comparative medical academic infrastructure.

**Dichotomy between Industry and Academia**

Commercial breeders of laboratory rodents have benefited greatly from scientific advances in rodent infectious disease diagnosis and control. As a result, rodents from most major commercial vendors are now microbiologically well defined. The profit margins involved in selling these animals for research have supported comprehensive infectious disease surveillance. In large part, this has been demanded by scientists in the biomedical research community who "vote with their pocket books." Likewise, the industrial sector, with its centralized control over staff and programs and its capital for investment, can effectively maintain rodent populations in an infectious disease-free state.

In contrast to industry, academic animal facilities cannot effectively maintain "clean" populations of rodents without considerable effort. Thus, the acquisition of "pathogen-free" rodents from commercial sources into academic animal facilities with enzootically infected rodents commonly creates opportunity for epizootic disease among immunologically naive, incoming commercial rodents that are exposed to enzootically infected academic rodents.
The situation is not necessarily a one-way exchange of pathogens. Commercial breeders are renowned for their selective definitions of “specific pathogen free,” so the buyer must beware. Furthermore, when an infectious disease breaks in a commercial rodent production colony, the buyer often is the first to know. In addition, commercial vendors, even though aware of the outbreak, may be reluctant to disseminate bad news that may inhibit sales. Vendors have also been faced with the emerging knowledge of a novel infectious agent that has been present, but undetected, in their colony, which becomes detectable with application of new diagnostic technology or new awareness of a pathogen. Murine parvovirus and Helicobacter infections are 2 very good recent examples. In addition, the breakdown and rederivation of a production colony is a significant operating expense for a profit-oriented organization. Thus, commercial and academic motives do not necessarily match. They can and do pose problems.

Changes in Biomedical Research Animal Use

Perhaps the biggest single issue facing rodent infectious disease control in the next decade is the burgeoning populations of genetically altered mice, with estimates of mouse populations increasing 20% per year in the nation’s biomedical research colonies. Techniques for transgenic manipulation and embryonic stem cell-based targeted mutations, including conditional, temporal, and tissue-specific mutations, are being made available to a wide array of scientists. The biomedicai research community is graduating from an era of molecular biology/single gene effects to a new era of “functional genomics” or “physiomics,” requiring analysis of gene function at the whole organism level. Specialists in rodent biology and infectious disease are needed for these transitions to avoid misinterpretation of phenotype. Across all institutes of the National Institutes of Health (NIH), there is global interest in integrative biology, and the laboratory mouse has become the preeminent model system. Major schemes are being considered for genome-wide chemical mutagenesis and the need of efficient methods for mRNA, protein, physiological, behavioral, and pathological phenotyping. Furthermore, single gene function is giving way to increasing interest in multigene interactions and complex genetic traits. The numbers of genetically altered mice are going to continue to expand at unprecedented levels for the foreseeable future.

Commercial enterprises cannot possibly supply these genetically altered mice to the scientific community, since the mice are much too diverse and the market is too small for profitable production. Therefore, academia will continue to be the site for their production, maintenance, and distribution. In the case of transgenic mice, virtually every line is unique, whereas site-directed mutants can be recreated. Nevertheless, the cost and time associated with creation and genetic stabilization by either method require continued maintenance of breeding colonies. A major need exists for cryopreservation of already created mutant mice, and although this approach may appear to be affordable, the time and money required to bring mouse lines out of the frozen state are significant.

In contrast to the not-too-distant past, when animal facilities were populated principally with a manageable number of commercially obtained, “pathogen-free” strains of rodents, animal facilities are now filling with a myriad of genetically unique mice, many with unpredictable or unknown immunological anomalies. These mice are being directly created by investigators, as well as extensively shared by investigators among institutions. The new generation of young scientists possesses little insight into biology and diseases of the mouse. Mice are commonly viewed simply as research tools, with little awareness of their infectious disease status. Indeed, a common problem arising in the scientific literature is misinterpretation of phenotype due to underlying infectious disease.

The foregoing series of articles is really directed at the very complex issues facing academia, rather than the much simpler situation in industry. The article by Jacoby and Lindsey (1998), which points out the ubiquity of a rather wide variety of pathogens in research rodents, accurately reflects the scope and magnitude of the problem. The survey data they present is a few years old, but more current data are likely to reflect an even more serious situation. Without effective infectious disease management, the entire national research enterprise is open to devastating epizootics or—equally important and much more common—misinterpretation of research data. Unfortunately, surveillance, quarantine, and rederivation programs, even if effectively in place, cannot keep pace with demand and the constant international traffic of genetically unique mice.

Opposing Financial Pressures

While forces are evolving for increased populations of mice and, to a lesser extent, rats, a number of financial forces have been working against that trend. Most notably, the NIH budget has suffered significant constraints in the last 10 yr or more, resulting in a lack of capital for investment in the academic research animal infrastructure. In brief, facilities are outdated and new construction has been under-funded. For a brief period, national research animal populations actually declined, and some institutions shut down animal facilities for much-needed research space. The net result of these factors is that existing animal facilities are simply incapable of handling the unexpected and burgeoning demand for housing large numbers of research rodents. As awareness of rodent infectious disease has increased, overcrowded animal facilities have relied more on “proximal” means of biocontainment to protect research rodents, such as filter-top caging, ventilated caging, or isolation cubicles. These methods often take up more, rather than less, room or make effective infectious disease surveillance more difficult because of the need to test at the cage, rather than the room, level.
Simultaneously, animal care operating costs have risen astronomically, and such costs have been passed directly on to users. US Office of Management and Budget (OMB) Circular A-21 is a govermental paradox that inadvertently discourages general support for animal programs (and animal welfare). Interpretation of OMB Circular A-21 by the Department of Health and Human Services and the Office of Naval Research has led to placing more of the cost of animal care directly on users (higher per diem charges). Institutions, which are also constrained by budgetary issues, are seldom willing to provide compensatory subsidies to animal programs since the dictates of Circular A-21 are invoked. The concomitant, increasingly onerous, and costly government-mandated regulations for animal care and use are unfortunately often arbitrary and not scientifically based. The net result is extreme pressure by investigators to reduce costs of animal care, and among the most vulnerable areas for program cutting are diagnosis and control of infectious disease.

Attrition of the Comparative Medical Academic Infrastructure

Finally, reductions in NIH funding in the recent past have also had a detrimental effect on academic programs that support rodent infectious disease research and technology development. The National Center for Research Resources (NCRR) dismantled its successful Diagnostic and Investigative Laboratory (DIL) funding mechanism. These DILs, awarded to several regional academic laboratory animal programs across the country, supported a fragile network of laboratories engaged in recognition of emerging diseases and development of new diagnostic technology. The diagnostic methods and many of the diseases mentioned in the preceding review articles were nearly all developed or recognized under the aegis of DILs. The current absence of these programs, or alternate programs, precludes critically needed discovery mechanisms for emerging diseases and development of efficient new diagnostic technology. In addition, despite the increasing total NIH budget, the NIH/NCRR Comparative Medicine Program budget has declined in the past decade, reducing the opportunity for veterinary scientists in these fragile academic laboratory animal programs to maintain critical mass for training and science that benefit rodent-related research. Finally, although commercial breeders and diagnostic laboratories have benefited immensely from this technology, they have returned very little to support academic programs in laboratory rodent sciences.

These combined forces of withering support have also had a detrimental effect on attracting veterinary professionals to academic careers. Potential talent in the laboratory animal professional community has been absorbed by the expanding regulatory roles demanded of veterinarians in biomedical research institutions. In addition, veterinary schools have deemphasized academic career training and have never emphasized the importance of laboratory animal sciences, despite the enormous economic impact of laboratory animals on our nation’s health and scientific enterprise. The laboratory animal veterinary community has now evolved as a largely service-related branch of the profession, with little academic luster. Thus, there is very little remaining talent to replace the aging generation of veterinary scientists who have traditionally spearheaded laboratory animal disease research and diagnostic development and who have directed or chaired programs that provided critical academic infrastructure to support both veterinary and nonveterinary laboratory animal scientists.

Renewed Urgency for a Comparative Medical Academic Infrastructure

A recent report by the National Research Council, entitled Biomedical Models and Resources: Current Needs and Future Opportunities (NRC 1998b), documents recognition of the need for expanded infrastructure to support the nation’s biomedical research enterprise. In addition, a panel of scientists (the Dove-Cox Committee) was convened by the NIH Director, Harold Varmus, to specifically define and establish priorities for the production of mouse genomics and genetics resources (Dove and Cox 1998). As a result of these reports as well as other planning forums, the NIH has renewed its commitment to investment in laboratory animal (and in particular mouse) biology. This investment includes scientific efforts in mutagenesis, cryopreservation, genetic mapping, as well as improvement of animal facilities and development of centers of excellence termed by NCRR “Comparative Medical Biotechnology Resources” that can serve the national infrastructure. Training initiatives in rodent biology have also been promulgated. Dr. Varmus has recommended the redirection of resources within various institutes toward this effort, with plans for expanding the NIH budget to specifically address these important issues. A number of targeted requests for proposals and program announcements have begun to appear before the scientific community. For the first time in many years, both houses of Congress and both major political parties recognize the importance of investing in biomedical research.

STRATEGY FOR FUTURE SUCCESS

Thus, these are heady times for Comparative Medicine and rodent biology, which provide new opportunity for addressing the very complex issues of rodent infectious disease diagnosis and control. Unfortunately, there is little left to respond rapidly and significantly to these initiatives. The laboratory animal scientific infrastructure is seriously atrophied and demoralized. The veterinary profession, with its own and even larger problems, must react immediately to seek, attract and train new students matriculating into veterinary schools who will track into academic careers. This requires faculty mentoring (which is lacking in laboratory animal sciences) and creation of training opportunities for
professional students. What the veterinary profession does in response to this overture from NIH will have an enormous impact on the future. If the profession fails to respond to this calling, veterinarians will quite frankly never again be the fulcrum for integrative biology, where they should be contributing, but will rather be relegated to a regulatory and policing function, where they are already headed.

Considering all of the above factors, the NIH should be encouraged to invest in the Comparative Medical Biotechnology Resource concept, but this approach will not solve the overwhelming need for a far more comprehensive Comparative Medical academic infrastructure that must be reconstructed beyond just a few centers of excellence. Funding is needed for applied research on diseases of, and improved diagnostics for, laboratory rodents. This would serve the dual purpose of responding to scientific need, as well as rebuilding the academic infrastructure that will be needed for creating the critical mass for training academic laboratory animal specialists and scientific programs. The rapid unraveling of the NIH/NCRR Comparative Medicine Program DILs and other research support has been devastating to the field of comparative medicine, and efforts must be made to redevelop and maintain a stable base of support for scientists who invest their careers in the comparative medical sciences.

Training is critically needed, but NIH initiatives fail to address the seriousness of the problem. Training must begin earlier in the training process to capture the talent needed. This involves expanded training opportunities for talented academically oriented students. For academic veterinarians, such training program funding must provide an incentive that encourages veterinary schools to aggressively seek and attract young people with an inclination toward academic careers, and to encourage emphasis of laboratory animals as mainstream veterinary species of significance. The targeted plans for advanced training of veterinary graduates to specialize in laboratory animal sciences is laudatory, but unfortunately, precious few individuals can be attracted to these programs at the present time. Commercial laboratory animal breeders should be encouraged to reinvest in this enterprise to reinvigorate a discipline that has traditionally benefited them. Unfortunately, most of the coming decade must be devoted to reconstruction of the discipline of Comparative Medicine and its academic infrastructure at a time when it is urgently needed.

Finally, the rising costs for instituting and maintaining effective rodent diagnostic programs that deal with the increasingly complex world of rodent research cannot be placed as direct costs to investigators. Animal health and welfare, not to mention protection of scientists from the adverse effects of infectious disease on their research animals, are unequivocally institutional and governmental responsibilities. Core funding to support rodent health programs must be shared by host institutions and NIH. OMB Circular A-21, although well intentioned, does not reflect an understanding of the importance of indirect cost support for both animal health and welfare and protecting the NIH investment in biomedical research.2 As long as these costs are considered direct costs for investigators, our goals to supply and maintain healthy research animals, to create minimal waste of animals, to maximize NIH’s biomedical research investment, and to ensure quality animal-based research cannot be achieved.

REFERENCES


Jacoby RO, Lindsey JR. Risks of infection among rats and mice at major biomedical research institutions. ILAR J 39:266-271.


2For another opinion, visit ILAR's Home Page (http://www2.nas.edu/ilarhome) and download Approaches to Cost Recovery for Animal Research: Implications for Science, Animals, Research Competitiveness, and Regulatory Compliance—Interim Report (NRC 1998a). The ILAR-sponsored Committee on Cost of and Payment for Animal Research, which produced the report, has moved on to identify efficient ways of caring for animals and using them in research. This second phase of the committee’s activities will depend on results of a survey sent to selected institutions. The final report is expected in late summer 1999.


