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September 22-23, 1976

INSTITUTE OF LABORATORY ANIMAL RESOURCES
DIVISION OF BIOLOGICAL SCIENCES
ASSEMBLY OF LIFE SCIENCES
National Research Council

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Preface

This volume contains the prepared papers and discussions of a National Academy of Sciences-National Research Council (NAS-NRC) Symposium on Laboratory Animal Housing. It was held at the Hunt Valley Inn, Hunt Valley, Maryland, on September 22-23, 1976. The Symposium was organized by a committee of the Academy's Institute of Laboratory Animal Resources, Division of Biological Sciences, Assembly of Life Sciences. The committee was comprised of an engineer, an architect-engineer, directors of laboratory animal facilities, and investigators in laboratory animal science and medicine. The purpose of the Symposium was to update and disseminate information on criteria for aspects of laboratory animal housing, including design of specific types of rooms; special facilities geared to particular research; the necessity for hazard containment; energy conservation and management; air-treatment and handling systems; automated feeding, watering, cage flushing, and central vacuuming systems; suitable electric power, acoustics, waste disposal, and construction materials. It was essential that the scope and complexities of housing be examined: The last Academy symposium on laboratory animal housing was held in 1963. Many questions raised at that meeting have remained unanswered, and new problems have emerged as well, necessitating a reassessment of information and priorities in the design, use, and management of animal facilities.

Because the conference was scheduled to last only 2 days, a limited number of topics could be discussed. At the outset, the organizing committee agreed that participants in the Symposium should consider:

- all animal housing, including research facilities;
- the general state of knowledge on laboratory animal housing;
- comparative costs of renovation versus new construction;
- major problems in existing facilities;
- opinions from users as well as designers of facilities; and
- cost-effectiveness and energy conservation.

Each topic was planned as a presentation rather than as a "how to" or "show and tell" exercise and was scheduled into sessions grouped according to compatibility of subject matter.

Speakers, of course, were chosen on the basis of their scientific expertise, and the selection of authorities on laboratory animal science,

engineering, and architecture was intentional. Many of the problems and challenges inherent in laboratory animal biology and housing can be resolved or met only through the collaborative efforts of professionals in diverse fields. The general approach to each session was to have a series of speakers in the medical and biological sciences define the problem and examine the scientific basis for considerations, followed by the introduction of architectural and engineering solutions when appropriate.

Although the Symposium did not answer all questions or cover all subjects, it clearly revealed that the state of laboratory animal housing is not one of excellence. The information presented--sometimes a lack of new information--seemed to emphasize that many commonly held beliefs, practices, and recommendations apparently are not based on empirical data, but rather are perpetuated by habit or tradition. Moreover, a distinct and urgent need exists for establishing and maintaining exchanges of information among engineers, architects, and laboratory animal specialists. However, the prospects for the future seem to be encouraging. Using the information at hand as a base, communication among all interested persons could assure not only discovery of new approaches and/or techniques, but also sound recommendations regarding problems peculiar to laboratory animal housing. An expert group of representatives from engineering, architecture, veterinary medicine, and laboratory animal science also could foster research in areas in which knowledge is limited.

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I

Animal Facility Management and Design in Terms of Performance— Update of 1963 Symposium

Have Animal Research Facilities Served the Purpose for Which They Were Designed?

ALVIN F. MORELAND

Before the end of World War II, the housing of laboratory animals was a simple process--or at least it was viewed as such by researchers at the time. Sophisticated methods of animal care were apparently thought unnecessary, if not undesirable. The intervening years have shown these notions to have been almost incomprehensibly naive. Although much sound animal research was accomplished before 1945, we are now uncomfortably aware of the necessity of reevaluating some of the older data because of the poorly defined and uncontrolled animal models then used.

Beginning about 1945, the amount of biomedical research being conducted in the United States began to expand at a rapid pace and the demand for laboratory animals correspondingly soared. These biological organisms, behaving as they are known to do, began to precipitate difficult problems in the management of husbandry and disease. All factors that could influence physiological processes or the spread of animal disease became recognized as factors to be dealt with by biomedical investigators, yet most scientists were poorly trained to deal with them. Therefore, because of their own experiences, researchers began to realize the complexity of the care of research animals. Concomitantly, specialists in managing research animals started to assume responsibility for animal housing. Thus, facilities for housing of laboratory animals became a matter of greater importance. This paper addresses whether or not the facilities constructed over the last 30 years have served the purpose for which they were designed. One would have to respond with a qualified "yes," but hasten to state that the fulfillment of objectives has not always been optimal nor as inexpensive as perhaps it should have been.

Gorsline (1963) went to the core of the problem with his description of what an architect must face in planning animal housing:

> It is precisely because research is a thing of change that the architect's job is especially difficult. New fields open, old ones close, and the emphasis migrates. The difficulty is intensely marked in university research, where turnovers in faculty and graduate school can alter aims in the course of a single academic year. The architect's dilemma is how to meet todays's demanding facility specifications, while leaving the way open for easy--often radical--modification of the building plan and operation. He must meet the architectural requirements of environmental control, isolation, and efficient care--elements provocative of design rigidity--and do so with a plan permissive of transformation into other plans not yet known.

If Gorsline had used the word "planner" instead of "architect" he would have reinforced one of the more striking conclusions reached during the Academy's 1963 symposium--that the design of facilities for research animals must be a *joint* endeavor embracing the architect, engineer, and fiscal specialist, as well as the animal health professional (usually a veterinarian) who will be responsible for directing the facility. Facilities conceived without the advice of a knowledgeable and experienced laboratory animal health professional who is completely familiar with the institutional research program have suffered major deficiencies in design.

MATERIALS AND METHODS

To answer the topic questions of this paper, I sent a questionnaire to directors of 40 large (medical centers and universities) and medium-sized (medical schools, hospitals, veterinary schools) institutions spanning 9 types of facilities; 37 institutions made useable responses. Forty institutions were thought to comprise about 15 percent of the institutions of this size, and their selection was made at random. This percentage of the estimated total was expected to yield results of good predictive value. Facilities were categorized according to their research program into 3 types, as set forth in Table 1.

Table 2 summarizes the time period in which the facilities answering our survey were constructed. It shows 89 percent of the facilities were constructed after 1950 and 70 percent of them after 1960.

SURVEY RESULTS

Knowledge of Design Criteria

The design criteria of these facilities were not readily available or were not known by the respondents. Only 7 reported general knowledge of the design criteria, which tended to be described in such generalities as "those guidelines in the *Guide for the Care and Use of Laboratory Animals*" (ILAR Committee on Revision of the Guide for Laboratory Animal Facilities and Care, 1972), "15 to 17 complete (100%) air changes per hour," "74°F ± 2°F temperature," "relative humidity (RH) 50% ± 5%," "provisions for positive or negative pressure," "85% filtration efficiency," or "impervious surfaces."

Centralized or Dispersed Facilities

Of the institutions in Category A, 7 reported wholly centralized facilities, 13 reported centralized facilities plus separate sites for special programs and multipurpose programs, 6 reported centralized facilities plus satellite facilities solely for special programs, and 3 reported centralized facilities with satellite facilities solely for multipurpose programs. The special facilities mentioned were for biohazard containment, surgery, nuclear medicine, barrier animal breeding, research on farm animals, or dog kennels.

TABLE 1 Types of Facilities Surveyed[a]

Category	Medical School	Medical Center	University	Hospital	Veterinary School
A	12	6	5	3	1
B	1	0	0	0	0
C	0	0	0	0	0

Category	Military Installation	Primate Center	Private, Commercial, or Govt. Laboratory	University Research Farm
A	1	0	2	1
B	1	0	5	0
C	0	1	1	0

[a] A = Multidisciplinary--multispecies--general purpose research
B = Multidisciplinary--multispecies--single purpose research
C = Multidisciplinary--single species or order--general purpose research

TABLE 2 Construction Dates of Laboratory Animal Facilities Reported in Survey[a]

Facilities Category[b]	Before 1950	1950-1960	1960-1970	After 1970
A	6	9	17	12
B	0	2	3	2
C	0	0	3	0

[a] The construction periods of some of the 37 facilities overlap the temporal divisions made above; hence some of the institutions are cited more than once.

[b] A = Multidisciplinary--multispecies--general purpose research
B = Multidisciplinary--multispecies--single purpose research
C = Multidisciplinary--single species or order--general purpose research

Success of Original Planning

To determine the accuracy of initial planning, institutions were asked if the projected usage had been realized: 21, 3, and 1 "yes" and 8, 3, and 1 "no" responses were obtained from the Category A, B, and C facilities, respectively. Some reasons for not realizing initially projected usage were:

- X-ray, survival surgical, and postoperative care facilities were not needed;
- biohazard facility had been insufficiently utilized; and
- too much animal space had been allotted.

For example, 12 Category A respondents indicated that total square footage formulas for the animal housing and support areas had proven accurate, whereas 16 indicated that they were inaccurate. Two reported square footages too large, and 14 reported them too small. One out of 6 Category B facilities reported the square footage formula had been accurate, whereas 5 indicated that facilities, as built, were too small. Two Category C facilities indicated facilities had been constructed too small.

Another aspect of the accuracy of planning can be measured by the necessity for renovation to meet new needs. To a question asking if renovations had been necessary, 19 A, 5 B, and 2 C facilities responded "yes" and 10 A and 1 B facilities responded "no." Most of those responding affirmatively indicated that the renovations had been difficult and costly, except for 4 A facilities and 1 C facility. The C respondent explained that renovation had been relatively easy because no internal load-bearing walls had been erected; the steel frame construction had allowed easy and inexpensive changes of room configurations. Two facilities used numerous small buildings of wood, metal, or concrete, making renovations or replacements inexpensive.

Veterinary Services

Nineteen A, 5 B, and 2 Category C respondents reported that they had originally planned for veterinary services. Ten A facilities and 1 Category B facility had not included them. Of the 11 reporting no veterinary service facilities in the original construction, 10 said that such facilities had been added. Five added them by expansion, 4 by encroachment on animal or laboratory spaces, and 1 by expansion *and* encroachment on animal areas. In addition, 4 facilities originally reporting veterinary service facilities indicated that these facilities had been enlarged, 1 by expansion and 3 by encroachment on animal facilities. This finding points up a serious problem--the inevitable shortage of animal housing areas when such conversion becomes necessary.

Expansion of Services

Since there is always a chance of incorrectly estimating the need for areas for animals, it is interesting to note that the possibility of expansion of services was only built into 13 Category A and 2 Category B or C facilities. Eight Category A facilities replied that this expansion was accomplished as planned, whereas 4 stated it was not accomplished as planned, but instead, new plans were developed and the expansion took place elsewhere. Two of the Category B facilities expanded as planned, whereas 3 B and 2 C facilities reported that expansion took place at other sites.

Conversion of Space for Animals

Frequently faculty bring to bear strong pressure upon the administration to convert space for animals into other quarters, primarily laboratories. Sixteen of the Category A facilities reported that such conversion had not occurred, whereas 13 reported such conversions, which involved percentages ranging from 60-75 percent to a low of 2 percent, with an average of 15-20 percent. All facilities noted that conversions were quite costly. Two Category B facilities reported a conversion of 30-50 percent of the animal space to other uses. Two A facilities reported that space had been returned to animal rooms.

Performance of Structural Materials

Nine Category A facilities responded that structural materials performed as expected, whereas 20 A, 5 B, and 2 C facilities reported that structural materials did not perform as expected: for example, epoxy floor coverings pitted or deteriorated (12); concrete floors cracked or pitted (5); wood or hardboard walls rotted (3); gypsum plaster walls deteriorated (1); quarry floor tiles lifted (1); 7.5-cm floor drains too small (1); cement block walls poorly sealed (1); chain-link wire on dog runs inadequate (1); plastic-coated acoustic ceiling tile unsatisfactory (1); transite ceiling tiles impossible to seal against insects (2); wooden sink cabinets rotted (1); wooden loading dock rotted (1); quarry tile hallways noisy with cage traffic (1); and nonwaterproof tile grout made it difficult to keep walls and floors sanitary (1).

Performance of Engineering Systems

Engineering systems were a source of diverse problems. Seven Category A, 2 B, and 2 C facilities reported that such systems as heating, power, air conditioning, ventilation, waste, and lighting performed to specifications, whereas 22 Category A and 4 B facilities reported they did not. Problems cited were: heating, ventilation, and air conditioning (HVAC) operated at less than 100 percent designed capacity (10); incinerator failure or malfunctioning (3); machinery too sophisticated for maintenance crew (5); unreliable thermostats and humidity sensors (3); inadequate electrical power (3); exhaust filters excessively clogged (1); ceiling-mounted vacuum-breaker water leaks (1); ventilation ducts too small (1); leaks through cracks in floor to rooms below (1); vacuum waste system inadequate (1); sump pumps serving flush drains clogged with animal hair in below-grade facilities (1); charcoal filters required too frequent maintenance (1); odors leaked from ventilation ducts (1); and incandescent lighting inadequate and expensive (1).

Ease of Adaptation to New Technology

Adapting older facilities to such new developments as more sophisticated caging methods, automatic flushing, automatic watering, pens versus cages, etc., proved to be difficult for 12 Category A facilities and 1 B facility. All of the remaining facilities replied that their design was modifiable. Most of the problems centered around adaptability to automatic flushing. The absence of floor drains or floors that were sloped to the drains created the principal trouble because installation of troughs was difficult or impossible. Floor drains in the center of a room and drains smaller than 10 cm in diameter also made automatic flushing difficult, impracticable, or impossible.

Performance of Special Machinery

Special machinery performed inadequately in 20 of the facilities, whereas 15 reported no inconveniences from it. Two facilities stated that no special machinery was used. Cage washers were noted to be particularly bothersome by 15 facilities, and autoclaves, special airflow cubicles, microbiological hoods, high-pressure washers, and constant-temperature rooms gave trouble in 1 facility each.

Value of Emergency Systems

Whether or not to install emergency systems in research animal facilities is a perennial question. Does the service justify the expenditures involved? Twenty-two Category A, 5 B, and 2 C facilities reported that emergency systems had been used or needed, whereas 7 Category A facilities and 1 B

facility answered that they had not. None of the respondents felt qualified to estimate any cost:benefit ratio of such equipment. The respondents commented: "used once in 5 years," "used once in 12 years," "used twice in 3 years for 20 minutes," "used 6 hours in 6 years," "used several times," and "emergency generator essential." One institution surveyed was merely equipped with emergency battery-powered lights, another was equipped to channel ventilation from other nearby areas by switching ducts, and 2 others had no devices except alarms, which were thought to be sufficient for their needs. Consideration of these responses would lead one to conclude that the need for emergency systems is rare and that the value of their immediate availability is a purely subjective matter. None of the facilities reported animal or human deaths that could be related directly to a deficient emergency system. However, one respondent in the New York area said that the 1965 "blackout" prompted the administration to acquire an emergency generator.

Other Physical Deficiencies

With the establishment in 1965 of the American Association for Accreditation of Laboratory Animal Care (AAALAC), more emphasis was placed on the need for care in planning and designing facilities for research animals. Although AAALAC emphasized both program management and physical plant facilities, it should be noted that in an analysis of deficiencies published in the AAALAC *Activities Report* (1973), 3 items directly relating to facilities design independent of management procedures were prominent: "Environmental control deficiencies" were cited 34 times, "improper storage facilities" 22 times, and "improper illumination" 12 times. Remarks about all these deficiencies also appeared in the results of the questionnaire. Such troublesome physical features should be subject to the scrutiny of the design and planning team.

SUMMARY

To determine if laboratory animal facilities have served the purpose for which they were designed, 40 large and medium-sized facilities of varying types were surveyed. More than two-thirds of the institutions were constructed after 1960, but physical obstacles that hindered the conduct of research had been built into the facilities nonetheless. Analysis of the questionnaire results indicates that more careful attention should be paid to provision of adequate living quarters and space for veterinary services in planning biomedical research facilities. Professional directors of animal resource facilities should be instrumental in their planning and design. The design of facilities should be geared to evolve with rapidly changing program needs. More than 40 percent of the respondents needed to initiate costly expansions and renovations. With proper design, much new or additional building could have been accommodated much more easily and inexpensively in the original construction. Several particularly troublesome construction materials were noted, as were difficulties encountered with engineering systems and special machinery.

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Does Management of Animal Facilities Complement Design Considerations?

C. MAX LANG

If we define management as the "directing, controlling, and supervising of any. . . activity with responsibility for results," the latter part of the definition--"with responsibility for results"--implies the desired relationship between design and operation. Ideally, this relationship should enable one to analyze, judge, and make subsequent improvements in both design and operation.

Unfortunately, a detailed analysis of the effects of design of housing for experimental animals upon subsequent management is rarely done. One explanation lies in the relatively small number of laboratory animal facilities in the country, and another in the shortage of managers qualified in the field of laboratory animal science. Since very few architectural or engineering firms design more than one animal facility, they seldom become expert in designing such facilities, nor do they know of available consultants in this field.

Qualified laboratory animal scientists are in short supply, and many of them view management as merely a business responsibility--one often given a low priority. That this attitude is not restricted to the field of laboratory animal science is evident from a statement that Tarrant (1976) attributed to Drucker: ". . . much of what we call management consists in making it difficult for people to work."

The rapidly increasing cost of animal research and the requirement for better-defined animal models emphasize the necessity of reexamining the relationship between the design and the operation of animal facilities. The quality of animal research should be made to conform to standards as high and as well defined as those applied to other aspects of the research project.

PROBLEMS IN OBTAINING DATA

The data in this paper were compiled from my notes on visits made since 1972 to animal facilities in 61 institutions located in 27 states. In all of these institutions, management is influenced by architectural design. This influence has been extremely variable, since nearly one-third of the facilities were old, and the rest were new, recently renovated, or still under construction. The sampling is admittedly inadequate. Because most of these visits were made in the process of reviewing research proposals, the information probably has a positive bias, in that it is derived primarily from institutions that have a large financial interest in the quality of data obtained from animal research.

In addition to inadequate sampling, another difficulty I encountered was related to the lack of written design criteria on which to base an assessment of operational management. In a few instances, the person currently responsible for managing the facility was also involved, to some degree, in planning it. Because involvement tends to reduce objectivity, any errors in planning were almost always attributed to someone else (the planning committee, the architect's failure to understand the intent of instructions, and so on).

In situations in which the designers and the managers of a facility had no relationship or interaction with the planning of their institution--whether because of personnel mobility or because a committee of users was responsible for the design--the managers made positive evaluations of those aspects of the design that worked well (whether or not they were planned to work that way) and negative comments on those that were less than ideal, even if the deficiency was a result of poor management rather than poor design. In

general, the better-managed facilities usually had some advice during the design stages from a qualified laboratory animal scientist. This superiority could be purely a reflection of that expertise, but it more likely reflects an administration that recognized early the need for such knowledge in both design *and* management. Correspondingly, facilities designed or managed without the oversight of an expert in the field were, in almost all cases, decidedly out of date. Such outmoded construction inevitably has an adverse effect on the biomedical research programs of the institution involved.

Most animal facilities surveyed were contained in a building designed for other purposes (teaching, research laboratories, or patient care). For about a quarter of the cases, the compromises were minimal and did not markedly affect operational management. In most, however, compromises that critically reduced operational efficiency were made. It was surprising how often expansion or renovation occurred without taking advantage of the opportunity to reevaluate the total program and correct its deficiencies.

Although this review contains too many variables and too small a sample to allow statistical analysis, the information obtained does provide a base for a discussion of the topic assigned: Does management of animal facilities complement design considerations?

CHANGING CONCEPTS OF DESIGN

The past two decades have seen many conceptual changes in the design of animal facilities. Barrier systems, for example, which were introduced in the early 1950's, were originally designed to keep animals free of germs, or at least of specific pathogens. Unfortunately, this effort has been relatively unrewarding in terms of research value. In some cases, the support services of management, faculty, or laboratory personnel were inadequate to ensure the success of the barrier system, or even to justify the existence of such facilities. Barrier systems are once again popular for specific purposes--e.g., biohazard containment or the provision of a closed, protected environment for housing delicate, genetic stocks. Their containment value is still questionable, since they are usually thought of as architectural and engineering systems without a concomitant concept of operation. Too frequently, the only common denominator in what constitutes barrier facilities is that of limiting access to the animals--and even that definition is extremely loose.

Another changing concept is that of the *clean-dirty corridor system*. Almost as many interpretations of this system exist as there are institutions that employ it. Four of the most prevalent concepts are listed below, in order of decreasing popularity:

- Anything goes. That is, a corridor has been built at either end of the animal room for entry and/or exit.
- Two corridors for personnel. One corridor is used by the animal technicians; the other, by the investigators. Although this concept is envisioned as a type of barrier system, the animal room, unfortunately, serves as a mixing area for personnel.
- Two corridors for storage. The clean corridor is used for the storage of clean cages; the dirty corridor, for those waiting to be cleaned.
- A controlled traffic pattern for all personnel and equipment, with periodic monitoring to ensure the absence of cross-contamination among animal rooms. Although very few of these facilities exist, it would appear that the concept of management can be made equal to the concept of design.

These varied management practices point out the lack of correlation between design and management. In general, the most popular, "anything goes" approach was most often found in facilities that did not have qualified managers. The more stringent program preserving the barriers, although less popular, was usually associated with highly qualified managers working to maintain animal quality and enhance operational economy.

A welcome change has transpired in the *relationship between the number of functions performed and the space available*. Many facilities built before 1960 tried to condense all activities into the available space. The more recently built, better-designed facilities recognize that it is better to omit units than to compress them into inadequate spaces (Harrell, 1974). These units can be added later when the need is pressing and funds are available.

In very few of the facilities was there any evidence that consideration was given to *operational costs*. Even the basic concepts of labor efficiency were ignored or rated relatively low. Although operational efficiency should be a primary topic of discussion in the construction of new facilities or in the renovation of old ones, it is very difficult to find much information on the subject. Another problem may be laid to the failure of the administration to recognize that the qualifications of an animal caretaker should equal those of technicians working in a research laboratory (Lang and Harrell, 1972). Mechanical systems and equipment--important as they are--are no substitute for qualified personnel.

Quality control is another changing concept, although it has many interpretations. The most visible aspect of quality control is the monitoring of mechanical systems. Although I attempted to demonstrate a correlation between the sophistication of monitoring systems and their effectiveness, I was unable to do so; i.e., there did not appear to be a correlation. I did observe, however, a tendency to rely too much on such mechanical controls. Too often it was assumed that heating, ventilation, and air conditioning (HVAC) control (and monitoring) of the secondary enclosure (the animal room) assured an environment of

similar quality in the primary enclosure (the cage or pen). In many cases, improvement in environmental quality by mechanical control was offset by poor management practices in the use of bedding, detergents, insecticides, filters, air fresheners, and other systems and aids that are known to alter the environment (Lang and Vesell, 1976).

Quality control may also include animal health programs, including a more subtle interaction between management and design requirements. Very few of the facilities I saw had an effective quality-control program for animal health. It is in this area that the specialty of laboratory animal medicine can make the greatest contributions, although it has had little impact in this field as yet, perhaps because of the lack of qualified personnel. Several facilities had adequate space designed for caring for animals but were unable to recruit qualified personnel; others had qualified personnel but lacked adequate facilities. Fewer than 10 percent of the facilities I visited had both adequate space and personnel. In these few cases, the benefits in terms of animal quality and operational economy were obvious.

The last concept to be considered for both design and management is acceptance by such users as investigators and technicians, etc. The consumer is primarily concerned about three items: availability of facilities and space, cost, and quality. The first two are relatively easy to evaluate. Quality, however, is very difficult to assess in terms of its influence on research data (Lang and Vesell, 1976). The animals' environmental requirements vary according to the research project, and the laboratory animal scientist needs to understand these requirements in the design *and* management of animal facilities.

IMPROVING THE CORRELATION BETWEEN DESIGN AND MANAGEMENT

The foregoing evaluation indicates that correlation between the design and the management of animal facilities is poor. How can it be improved?

The Need for Experts in Both Fields

Perhaps the first step is for each institution to commit itself to obtaining the counsel of experts in both design and management. It should be recognized that an individual who is an authority in one of these fields is not automatically an authority in another. Many superb managers of animal facilities are simply unable to envision their program in such a way that it can be translated into architectural design. Similarly, building consultants, regardless of their specialty, should not be expected to prepare a program of management that complements their design. Imperfect correlations of design and management can be minimized if the program director of the facility is included in all discussions relating to its renovations or additions. The manager is sometimes consulted either sporadically or very late in the design stages. As a result, he or she may overreact to the suggested solutions without fully understanding the total program or necessary limitations. Similarly, because of pressures of scheduling, the design team may be too quick to accept or reject answers without fully understanding their rationale.

The Need for a Definite Program of Operation

Until a definite program of operation has been established, attempts to design a suitable facility will be meaningless. Each animal is a sensitive biological system whose response can be altered by minute changes in its environment. The first phase of the design program is to identify those conditions that are necessary for the animal's comfort and to reduce the possibilities of environmental variables. Next, requirements of the research should be considered, with careful attention paid to any that may affect the animal's environment. Compromises may be necessary, but they should be minimal. It is only after completion of this planning that organic design features can begin to be formulated for space requirements and traffic patterns.

Space requirements depend largely on the type and magnitude of ongoing and future research. The amount of space needed for conventional and special housing should be determined by someone who is able to balance the demands of the research program with the requirements of the animals. (Examples of special housing include cubicles for isolation and facilities for biohazard containment.)

The requirements for animal space must be determined before one can estimate the space required for support systems: laboratories, service facilities, and administrative offices. These decisions must be made largely on the basis of the proposed operational program. Attempts to formulate a ratio between space needed for animals and space needed for support have been unsuccessful because each facility must be assessed thoroughly according to its particular operational program. Designs based on facilities existing elsewhere should be avoided, unless the two institutions have comparable programs. Even in such cases, individual improvements should always be sought.

After requirements for space have been determined, the flow pattern and spatial relationships must be established. The flow pattern probably has more impact on operational economy than any other aspect of architectural design. A knowledge of program requirements--types of movable equipment, especially cages, and any special items--is essential to a well-designed flow pattern.

Compromises in the design of animal facilities, based on cost:benefit ratios, are inevitable. The interaction between an animal and its environment has many effects on the research data. Since it is uneconomical, if not impossible, to control all these effects, priorities will have to be established by a reexamination of past policies and procedures to determine if they are

based on scientific principles. The cost of operating an animal facility is extremely high--it exceeds construction costs every 6 to 10 years --and any increase in mechanical controls will raise it.

Because the animal facility is really an extension of the research laboratory, the requirements for quality and control of variables are the same. The actual space needed for holding animals is only one consideration. Of equal importance is the space required to monitor the animals and assess their quality. Adequate professional and laboratory support can lower the cost of research by reducing the number of animals needed to obtain statistically reliable data. Such support depends on the design of a facility as much as it does on the number and qualifications of professional and technical personnel. Careful planning and commitment to quality will pay immediate and long-range dividends.

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Centralized Versus Dispersed Animal Care Facilities

ALBERT M. JONAS

In 1961, I joined the faculty at Yale and immediately became embroiled in discussions questioning whether animal facilities and/or management of animal facilities should be centralized or dispersed.* This question is still a fundamental issue at many institutions. Over the past 15 years, I have had the good fortune to serve on committees of the Institute of Laboratory Animal Resources, the American Association for the Accreditation of Laboratory Animal Care, the American Association for Laboratory Animal Science, and the National Institutes of Health, and I have visited a large number of biomedical research facilities of industrial and academic institutions. In addition, the Yale facilities have now grown to encompass over 7,804 m^2 of net animal space within the medical center and an additional 3,252 m^2 of net building space on 2 research farms. These visits and expansions have given me the opportunity to evaluate concepts in design and management on a continuous basis. My comments on this subject are based on these experiences and reflect my own biases for, unfortunately, the answers we seek may at times be more a function of art than of science.

Academic politics, the power struggle among and within departments, personal whims or deep convictions based on fact, fancy, or ignorance may bear on final design and management. The composition of the animal care committee, the knowledgability of senior administrators about animal research, and the priority they assign to animal research are key factors. Finally, the advice of a veterinarian and the degree of respect and credence his remarks carry will undoubtedly affect final decisions. However, veterinarians with extensive training and experience in laboratory animal medicine are, of course, not without their own personal biases.

Managerial concepts, as well as facility design, must be considered when we use the terms *centralized*, *decentralized*, or *dispersed*. If we were to pick one glaring problem frequently encountered by consultants, it would be the lack of understanding by animal care committees and architects of managerial concepts and methods of implementing programs. Facility design must reflect the stated requirements of individual investigators and lend itself to good management practices. The purpose of animal facilities is to provide space, equipment, personnel, and programs that allow and encourage, at reasonable cost, high-quality animal research. Facilities that are economically managed at the expense of investigator access and scientific productivity are undesirable. As in most endeavors, compromise and balance are essential for a successful solution.

THE PHYSICAL PLANT

The location of animal facilities within the institution is the primary point of reference for most discussions on centralized versus dispersed facilities. The disputes over who should manage these spaces and whether they should conform to an overall animal care program fill committee rooms. Factors promoting science may conflict with political considerations resulting in disruptive power struggles. It is important to dissect carefully these arguments into their

*In this paper, *dispersed* facilities will usually imply multiple animal research spaces at different locations, but under *central professional management*. Occasionally, the term will be used to indicate a decentralized facility and management.

respective positions--scientific productivity and academic politics. Advocates of central facilities are not always known for their objectivity, and investigators wishing exceptions are not always seeking irrational control. Thus, the final decision and "master plan" will reflect personalities, historical precedents, ongoing and future research programs, and the willingness to seek and listen to expert advice.

Extrainstitutional Factors

Another factor that influences management and design is the involvement of granting agencies and the government in setting guidelines for adequate animal care and enforcing "good laboratory practices." In addition, certain state and federal laws apply to animals used in biomedical research. Noncompliance of individual investigators could adversely affect other grantees in their institutions. Therefore, institutional as well as peer pressure to comply with legislation is strong. Investigators are further constrained by the Occupational Safety and Health Administration (Bureau of National Affairs, 1971) and regulations for facility design and utilization. Guidelines are now available for recombinant DNA experiments (National Institutes of Health, 1976) and for use of hazardous agents (National Communicable Disease Center, 1970), including oncogenic viruses and chemical carcinogens (National Cancer Institute, 1974, 1975). Fifteen years ago, few envisioned such extensive regulatory action by outside agencies, but changes in research direction and societal pressures required modification of programs and facilities. These alterations permit us to evaluate how past concepts of design and management have been adapted to these new requisites.

EVALUATING THE INSTITUTION

In order to assess centralized and dispersed facilities, I will propose a set of assumptions for a hypothetical animal research facility.

- The institution has at least 20 principal investigators using research animals.
- The overall quality of research is such that investigators require and demand a good animal care program.
- A minimum of 1,400 m^2 for net animal space and support facilities has been planned for or made available.
- Financial support is not the limiting deciding factor.

The first step in our evaluation is to ascertain the size of the physical plant and how its space is distributed; the number of investigators and their distribution vis-à-vis their laboratories, offices, and distance to clinics if applicable; the types of ongoing and projected animal experimentation; the number and kind of facilities for special uses (e.g., surgery, hazardous agents); and the type of animal species, daily census, and annual usage. Other factors to evaluate will concern rural or urban location, the degree to which access should be controlled, availability of central services such as chilled water and steam, and internal transportation routes such as service corridors and tunnels.

Next, the service functions and areas of the planned or operating animal care program must be divided into their major components, as illustrated in the following example:

Animal holding space (for rodents, rabbits, dogs, cats, primates, and large animals): for short-term research utilization; long-term research utilization; and quarantine.

Special facilities: for inhalation studies; chemical carcinogens; biological hazards; surgical facilities; postoperative care; radiological facilities; and radioisotope facilities.

Veterinary support areas: for treatment; postoperative care; necropsies; microbiology laboratories; clinical pathology laboratories; offices; and storage.

Other support facilities: for administrative offices; loading and discharge docks; cold rooms (animal and waste disposal); freezer space; volatile and hazardous agent disposal; storage space for food, bedding products, active cages, and nonactive cages; sterilization; washing and sanitation; locker rooms, luncheon areas, toilets, showers; and transportation corridors.

The third step is to estimate the dimensions of the primary components, and match them with the geographic location of investigators who will use those facilities. This coordination is the most critical and complex of the tasks involved because it determines the *feasibility* of dispersion. The next area of study concerns special requirements that necessitate intermittent monitoring of animals during an experiment and/or use of nonportable special laboratory equipment. A further complication exists if this equipment is used for acute and survival animal experiments.

The Investigators' Viewpoint

Investigators frequently desire complete control of their research laboratories and animal quarters, prefer unique space for their particular needs, and request maximal space to accommodate peak work loads. They prefer research space adjacent to their offices and look with a jaundiced eye on any program that tends to limit their ability to make independent judgments or that may result in increased operational costs. Questions raised by investigators using animal research facilities indicate their concern about centralization. They believe that the following issues should be adequately resolved: quality of research space and suitability for their research project, proximity to primary laboratory space, ease of entry and egress, and proximity to clinics and offices. In addition, investigators may fear that an unresponsive central bureaucracy, headed by a veterinarian, will be established. Such an

administration may be incapable of managing the special facilities or animal colonies. As in most professions, there are poorly trained and well-trained individuals. It is most unfortunate when the fear of having a poor-quality veterinarian in charge dictates the direction of the animal care program. Application of appropriate checks and balances in the institution should overcome deficiencies in personnel.

To determine *feasibility* more precisely, the above interests should be weighed with the following factors.

- Ability to provide quality animal care consistent with experimenters' needs and institutional policies.
- Logistical ability to support dispersed facilities, i.e., transportation of animals, supplies, and wastes; cage or equipment sanitation/sterilization.
- Acceptable cost:benefit ratio--the overall benefit for investigative research should weigh heavily in the equation.

In summary, to consider instituting dispersed animal research facilities, the following conditions must be met:

- A desire and need as perceived by investigators and/or management to have components of the animal research space located in more than one geographic area.
- Facility size and utilization are justifiable in a cost:benefit analysis.
- The quality of space and program will meet or exceed institutional standards and all appropriate governmental regulations and guidelines.

Investigators are interested in the final product--high-quality spaces, animals, and programs. The professional management team, veterinary clinicians, and animal health laboratory personnel also wish to provide high-quality environment and animals, but since in a properly run program they are charged with the responsibility of supplying this service, they look at the problem from a different perspective. Features that are most desirable for the users may in some instances be incompatible with the objectives of those delivering the service.

Laboratory Animal Management's Viewpoint

A facility located in one geographic area, encompassing all necessary services and spaces, and having adequate access control has decided advantages. Maintenance is easier and elements of physical plant design--air conditioning, heat recovery systems, lockers, and shower locks--may be superior. Support services such as washing facilities, sterilization areas, and feed and bedding storage may be used more economically. Monitoring of systems and personnel, access control to and from the facility, receiving and distributing animals and supplies, and disposing of wastes are all more efficiently handled. The design and utilization of special departments such as surgical, radiological, and radioisotope services may be far superior to their counterparts in small facilities. Duplication of expensive equipment (e.g., monitors in intensive care and surgical units, X-ray equipment, sterilizers, rack and tunnel washers) is unnecessary. Instead, more sophisticated equipment can be purchased.

Assignment of animal holding and research space and scheduling of support areas can be apportioned expeditiously; utilization and flexibility usually are superior in central facilities. Dispersed research support facilities may have several underused and overused services. Carefully designed central space can eliminate some of these weaknesses and make a variety of systems available to the investigator, ranging from germ-free research space and containment for hazardous agents to barrier systems and contiguous laboratories (Jonas, 1965).

However, even from management's viewpoint, dispersion has advantages in certain instances. Rodent breeding colonies should be as isolated as possible; facilities for hazardous agents should be situated for maximum control and for isolation from animal and human populations; primates may be handled better when they are separated from the main animal holding space; large animals such as swine, sheep, horses, and cattle may be best situated in rural quarters that have appropriate support facilities at the farm as well as within a central facility. Large inhalation studies may require sophisticated equipment and animal holding and support laboratories that might be best situated in a satellite facility.

In summary, management typically makes a strong case for centralized facilities because they provide the best space and equipment utilization for the least money, and usually permit a wide range of available services, animal holding space, and research opportunities for the scientific community they serve. Centralized facilities tend to encourage better personnel supervision and management, more efficient deployment of manpower, and a reduction in operating costs. If properly managed, they will have more uniform, superior animal care, as all units are on constant view to supervisor and staff.

ARRIVING AT A DECISION

Three main determinants in the decision to create centralized or dispersed facilities are:

- The investigators' desire for control of space and the total program, maximum accessibility, and scientific productivity.
- Management's desire for control of the animal care program, efficient design, and ease of daily operation with maximum cost savings.
- Academic politics.

In older, established institutions with multiple research buildings, clinics and a hospital, and a history of building dispersed animal facilities, the decision is obvious. In these institutions, elimination of all satellite facilities and the creation of one central facility is fre-

quently untenable for the investigators. However, a primary facility with regional units is usually acceptable. Regional or dispersed facilities in these older institutions generally include surgical suites, special research laboratories, and special animal holding space. Academic politics and investigators' desire for control frequently win the argument for retaining some of these satellite support facilities. If they are well run, interact with the professional veterinary staff, and meet the standards of the *Guide for the Care and Use of Laboratory Animals* (ILAR, 1972), then these support laboratories can be very effective. Animal holding facilities, however, should only be allowed in areas large enough to provide full services, including washing and sterilizing areas; cold rooms; discharge and receiving areas; adequate storage; and lockers, showers, and toilets for animal technicians. I have chosen--with some reservation--a minimum net figure of 450 m^2 for such a satellite facility. A smaller area yields such an unfavorable cost:benefit ratio that it is difficult to justify. Small, improperly serviced animal holding areas, which often result in questionable animal health and environmental control procedures, should strongly be discouraged. Ease of access to low-quality animals is usually not a justified stance. This is not to say, however, that small animal areas cannot provide high-quality animal space and research animals. Management and logistics problems, as well as costs, are usually high, but extenuating circumstances may justify their existence.

Smaller institutions or those establishments planning new research and animal facilities have the advantage of maximizing central core facilities with due consideration given to access by investigators. Serious thought should be given to master planning in all institutions.

Expansion of animal facilities should be instituted in concert with expansions of research facilities and programs. However, it is most difficult to enlarge central facilities at the same time a new research building is being constructed. Coordination of such projects is most difficult because of funding constraints and priority assignments. If a design and management concept is accepted that considers creation of additional secondary or dispersed facilities within new research quarters, then expansion of animal quarters can proceed in an orderly fashion. As new research facilities are created, animal quarters that complement the overall animal care program are added and space close to investigators is provided. This approach does not solve all logistical difficulties, because investigators may still, under certain circumstances, have to work at the central facility, or, researchers near the central facility may need to use the subsidiary facility if it has special equipment or features. A reasonable figure for animal space would appear to be 10-20 percent of the planned research space, i.e., a building with 9,000 m^2 could have 7,650 m^2 devoted to research and offices and 1,350 m^2 for animal support areas. To recall my previous statement that the minimum space necessary for sophisticated animal quarters should be a net 450 m^2, this estimate implies a research building with a minimum net research space of 2,250-4,500 m^2.

CONCLUSIONS

Centralized versus dispersed facilities should not be construed as a mortal battle between good and evil. Experience has clearly shown that poorly managed central facilities are just as bad or potentially worse than poorly managed facilities with dispersed units. In the latter case, at least some of the facilities may have superior programs. In order to decide which system is preferable for a particular institution, careful evaluation of existing and planned facilities is necessary, with consideration given to location of investigators and their type of research (e.g., aging, acute studies, inhalation, or hazardous agents). The quest is for a solution that will best provide a high-caliber animal care program most conducive to high-quality research. One must not lose sight of the individual investigator in this evaluation; his or her special needs must be addressed and suitable architectural solutions reached. Research programs change over the years, and fortunately animal quarters lend themselves to renovation and redesign, as also do laboratories and offices. All is not lost if a regional animal facility no longer serves its original purposes. It is to be expected that animal facilities will age and new technologies will replace the old. Regional facilities clearly lend themselves to improvement and modernization at feasible project costs. Massive central facilities have the decided disadvantage in that they tend to age overall, and modernizing may be postponed because of the high expenses involved, the disruption of many investigators, and possible consideration of relocation to a new modern facility.

If there is any answer to the problem of "centralized versus dispersed facilities," it is to create a major facility that can, if necessary, support additional autonomous regional facilities (that is, with independent washing, storage, and support facilities) *under central management*. The regional facilities should complement the core unit, and unnecessary duplications should be avoided. The ability to expand or reduce any of the facilities should be considered. In large institutions, a central core with additional dispersed facilities, each with a minimum net 450 m^2, may be construed as a positive rather than a negative planning feature. The added initial cost may pay dividends in terms of investigators' acceptance of the plan and future expansion and renovation programs.

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A Theory of Architecture: The Orchestration of Information

S. JAMES GOLDSTEIN

Architects and engineers are primarily organizers of information: They "put it all together." To be effective professionals, they must, of necessity, develop a high level of collaboration with the facilities managers. Having consulted for various biomedical programs and designed 2 medical schools, and having visited 65 academic medical centers in the United States, Canada, England, and Scandinavia for the past 14 years of my 30 years as a professional, I feel that I am a part of the bioscience family. As a member of that family, I speak from the inside. I also speak as an architect, as a structural engineer, and as a mechanical engineer, all parts of the design professions concerned with the man-made or built environment.

IDENTIFYING BASIC CONSIDERATIONS

The perceived increasing complexity of environmental design considerations (witness the holding of this symposium) for animals, as well as for humans, requires the generation, organization, retrieval, and dissemination and application of information--that is, the orchestration of information. I am not arguing for or against the use of computers, either in bioscience research or in architectural design. Rather, I believe that the interior architecture of a building (as illustrated in the figures that follow) should be an effective management tool over the life of that building. Accordingly, management's emphatic articulation of its requirements is the paramount determinant of planning and design decisions in our era, whereas in earlier cultures societal values were the paramount determinants; these values were stated by Vitruvius around 2000 years ago and by Sullivan circa 1893 to 1924.

In order to deal (whether manually, or by programmed processor) with information, it should be classified into categories (but *not* hierarchies) or, in mathematical terms, sets and subsets. This first step in organization immediately reduces the manipulable quantities of information to a fraction of the total. What informational sets are useful in this planning scheme? Is it justifiable for a manager or space designer to deal with categories, classes, or sets rather than aggregate raw data? Here is the nub of the manager's or the designer's problem: What information does he or she deal with, at each stage in the management or design of laboratory animal environments, and in what sequence of stages? Indeed, does an identifiable process of design exist, and is it the same for all projects and all designers? These fundamental questions are worth answering as best we can, with the tools at hand. The following lists enumerate the increasing complexity of the considerations involved, based on operative societal forces.

Scientific Considerations

- Increased use of sophisticated instrumentation in biomedical research.
- Increased use of multidisciplinary approaches in the organization and execution of biomedical research.
- Finer and finer discrimination of subatomic particles and of biochemical behavior at the genetic level (Handler, 1970).
- Increased concern with environmental hazards in biomedical research.
- Increased differentiation among environmental controls within biomedical research environments.

- Increased use of electronic data processing in research.

Construction Considerations

- Impact of worldwide inflation on the costs of labor and materials.
- Impact of inflation on the costs of money used for financing construction efforts.
- Relatively declining or "flattened" productivity of construction labor.
- Greater proportions of total construction costs devoted to mechanical and electrical systems.
- Increased emphasis on management and scheduling techniques to reduce the time of design and construction.
- Increased use of electronic data processing by construction management.

Design Considerations in Architecture and Engineering

- Increased use of controls and barriers in research environments, leading to finer differentiation of functions in assigned spaces and producing greater individuality and specificity in the aggregate animal facility of each institution.
- Increased use of open-plan, undifferentiated, modular, multipurpose interdisciplinary research laboratory environments. (This trend is noted as a countervailing yet parallel movement to the preceding item, because it appears to augur increasing generality of research facilities when control of biohazards is not an issue.)
- Increased sophistication of building instrumentation for environmental control and for energy conservation.
- Increased intensity and sophistication of energy conservation efforts, applicable over the projected life of the building.
- Increased application of multidisciplinary team-design concepts to major and complex projects.
- Increased availability of new technologies in materials, equipment, and systems of construction.
- Increased use of cost:benefit analyses and life-cycle costing to select materials and components.
- Increased use of value engineering* concepts for saving money and time.
- Increased use of electronic data processing in preliminary information management and in actual design of physical facilities.

Let me emphasize the very special new perceptions regarding the interdependence of design and construction. During the past 15 years, the results of all the foregoing construction and design considerations have had a notable influence on the following types of large-scale, and often unprecedented, man-made projects: airports, dams, interstate highways, major research complexes for governments, universities and corporations, missile systems, new towns, sports complexes, urban renewal projects, and the tallest skyscrapers (such as the John Hancock Building and the Sears Tower in Chicago and the World Trade Center in New York). As a result of these recent precedents, the architectural and engineering professions are now inextricably committed to organizational strategies relating to cost and time considerations, from the initiation of project planning, through the design and construction phases. Management and design are thus beginning to be seen as inseparable entities.

*A systematic, analytical approach to producing the highest yield from capital investment.

THE ORCHESTRATION OF INFORMATION

To return to the designer's need to understand information management and control, one may begin to find answers by considering a progression of categories of biology-based or operational information available to the facilities designer:

A. The animal
B. The animal environment
C. The cage
D. The animal holding room
    1. Differentiated or generalized for species
    2. Differentiated or generalized for function
    3. Differentiated by biohazard containment
E. The (virtually autonomous) isolation suite
F. The ancillary spaces
    1. Differentiation by function
    2. Variability of function
        a. Based on management techniques
        b. Based on technological instrumentation
G. Performance of operations: bringing research to the animal or the animal to the research site

Introducing concepts of space and environmental design that affect the foregoing considerations, we add the following categories:

H. Cage sizes and arrangements of racking
I. Density of animal occupancy
J. Room sizes, including lengths, widths, and heights
K. Compartmentalization whether fixed, modular, or flexible
L. Constraints of externally imposed physical forms and structures (illustrated in Figure 1)
M. Arrangement of spaces (illustrated in Figure 2)
    1. Differentiation between "served" and "servant" spaces (concepts attributed to the late Louis I. Kahn, architect)
    2. Zoning by area or use
    3. Grouping by species or by operations
    4. Simple linear or complex arrangement of rooms
N. Traffic or circulation, including the evo-

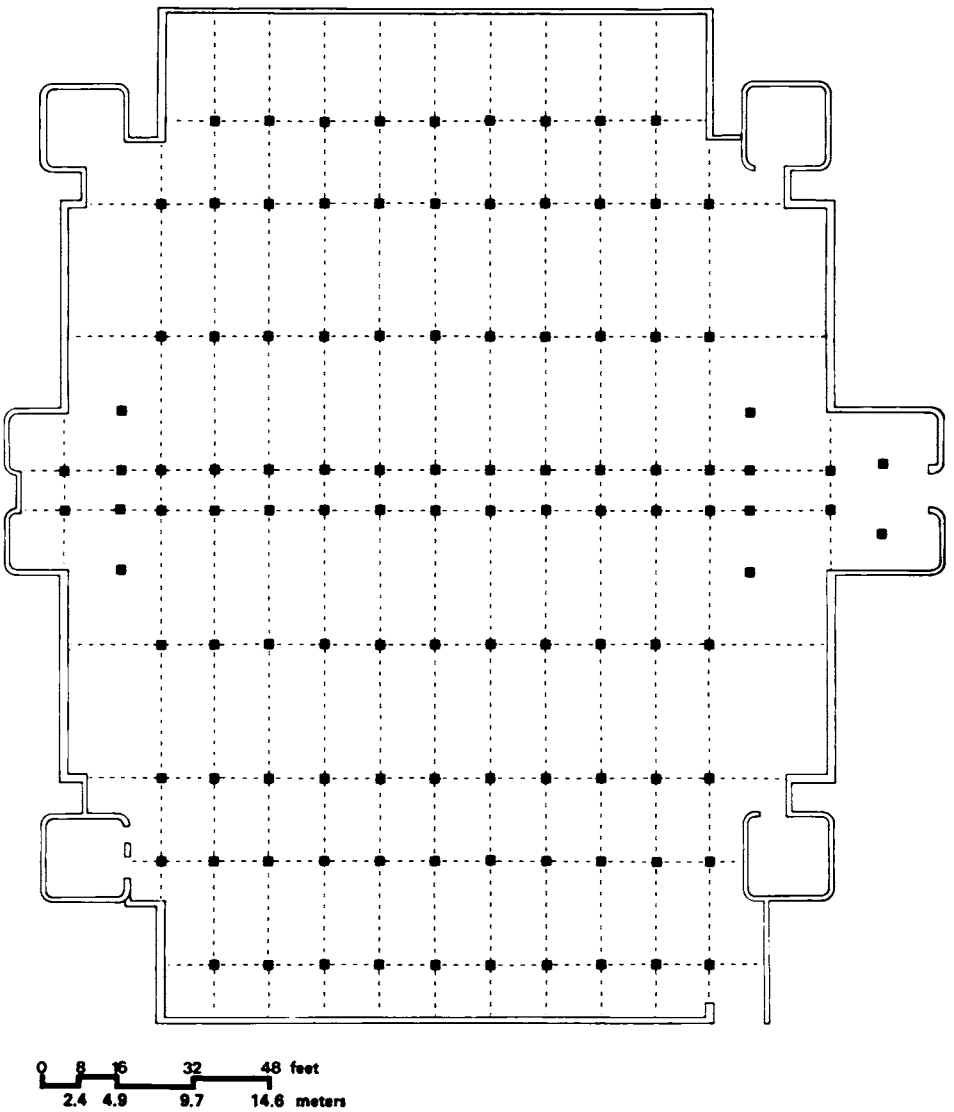

FIGURE 1 Structural grid.

lution of materials handling and other operational activities such as food, waste, and water distribution, cage cleaning, and clinical treatment and observation

O. Space-use efficiencies, measured in 2, 3, or 4 dimensions

P. Corridors (illustrated in Figure 3)
   1. Fixed, permanent location, with assignable space fronting same
   2. Flexible location, in field of assignable space with or without fixed points or nodes

Q. Service utility distribution (geometries for liquids, gases, power, and communications; illustrated in Figures 4, 5, and 6)
   1. Overhead configurations
   2. Underfloor configurations
   3. Core configurations
      a. Linear-horizontal arrangements (Often distributed over corridor ceilings, but sometimes run directly over ceilings of animal rooms)
      b. Linear-vertical arrangements (Known as "service closets"; originated at Bell Laboratories in Murray Hill, N.J., about 40 years ago)
      c. Spatial arrangements, serving back-to-back modules, both vertically and horizontally (Often called "service distribution galleries"; independently pioneered in this country by the author and by the late Eero Saarinen)
   4. Interstitial arrangements, a separate "mechanical systems" story, accessible to occupied floors above or below

R. Vertical circulation
   1. Stairs or other safety connectors
   2. Elevators

S. Horizontal tangencies
   1. Access routes
   2. Egress routes

T. Societal constraints, typified by:
   1. Mandated standards for animal care

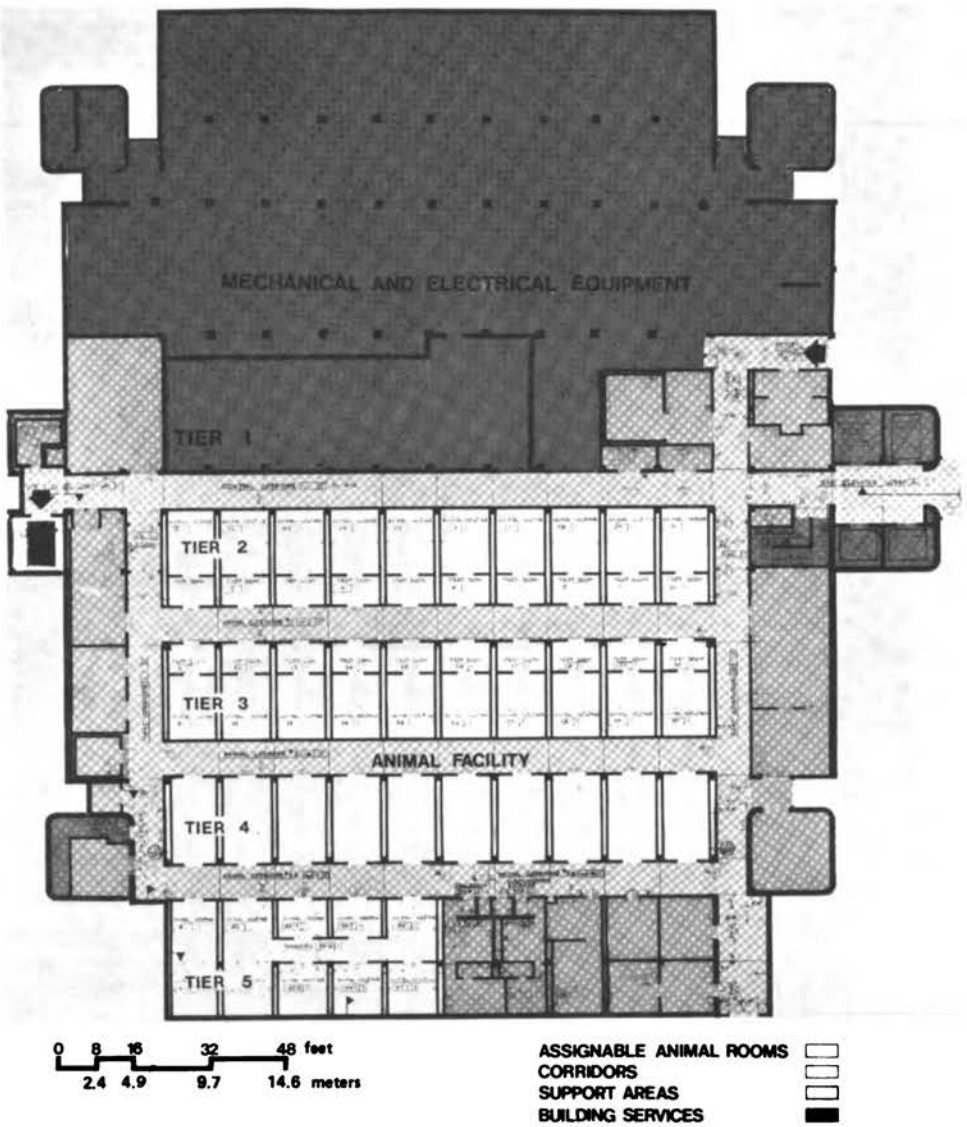

FIGURE 2 Occupancy plan.

2. Mandated standards for occupational health and safety
3. Mandated standards for physical safety in buildings

For the sake of simplicity, this outline has concentrated solely on elemental functional categories. Yet note how the branching possibilities on the list expand exponentially in their potential permutations and combinations. Hence, organization and discrimination are necessary to make effective use of this information, because the introduction of some counterproductivity, ambiguity, compromise, interpretation, uncertainty, or paradox is inevitable. Moreover, each facility manager and each facility designer will intentionally and unintentionally contribute their own emphases, concerns, and biases. And, over the life span of a new or remodeled facility, some activities will grow at the expense of others and adaptation to new conditions will follow.

It is not possible to provide a formal order or organization of information without arranging it in a hierarchy: a random gathering of information may be helpful, but it is not eminently valuable to the user, whereas a focused organization of information, the theme of this paper, is an essential resource. The value judgments suitable for ranking a given situation may be provided, derived, sought, or achieved by: analyses of precedent, acceptance of prevailing wisdom, felt necessities, feedback from experience, local constraints, operational analyses and syntheses, statistical analyses and syntheses, technical literature and information systems, regulatory legislation (or local interpretations of it), and performance evaluations. Feedback from performance evaluations is essential for substantive and statistical (operational) insights; institutionalization of such feedback is typically lacking. Especially strong linkages ought to be forged between the academic bioscience communi-

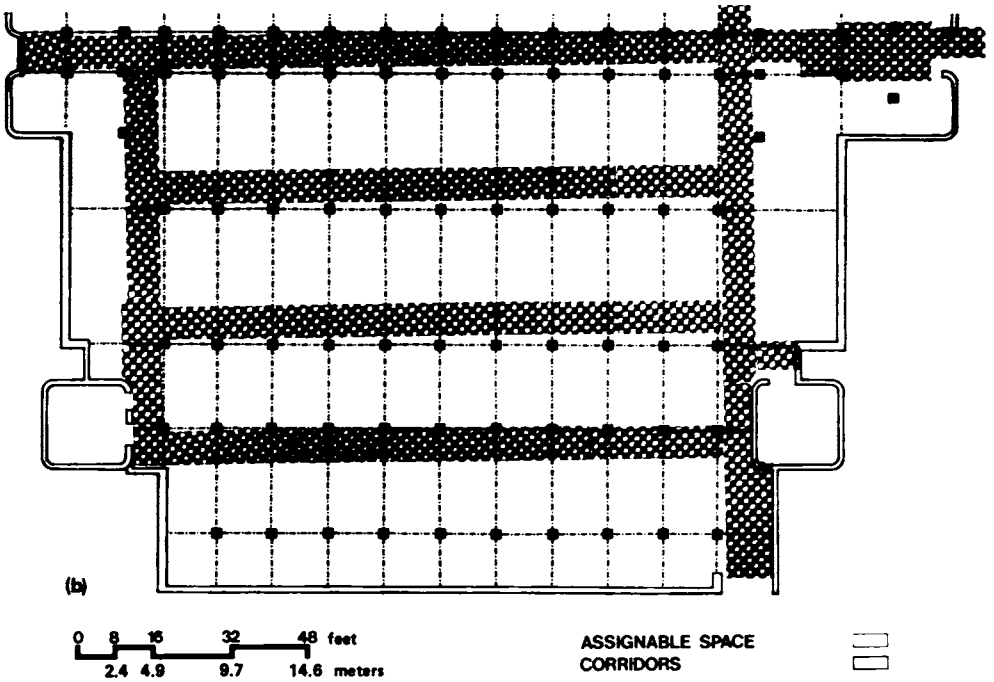

FIGURE 3 Circulation plan.

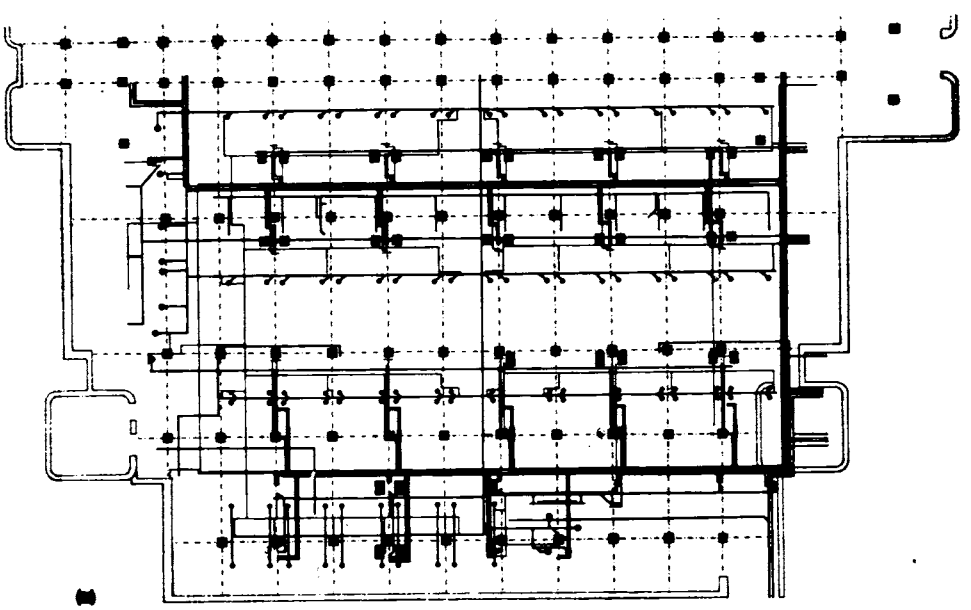

FIGURE 4 Plumbing plan.

ties and the schools of architecture in this country, because they can make many contributions to each other.

When your own priorities have been established, and your hierarchies ordered, you may be tempted to succumb to a simplistic form of determinism at one extreme, or an insufficient articulation of an organizing theme at the other. Historically, laboratory animal facilities in this country generally have been designed around the very simplest of closed geometries. It is time to consider a newer planning concept: "open geometry." Consider the effects of change upon extant facilities. No matter how the change was imposed --be it caused by technical obsolescence or programmatic modification or growth--the environmental consequences will include regrouping, relocation, subdividing, recombining, renovating, remodeling, or alteration.

In the past, most animal facilities have been slowly transformed by gradual physical changes; however, the *pace* of change has begun to quicken demonstrably. "Uniformity" and "standardization" are concepts that could never be applied success-

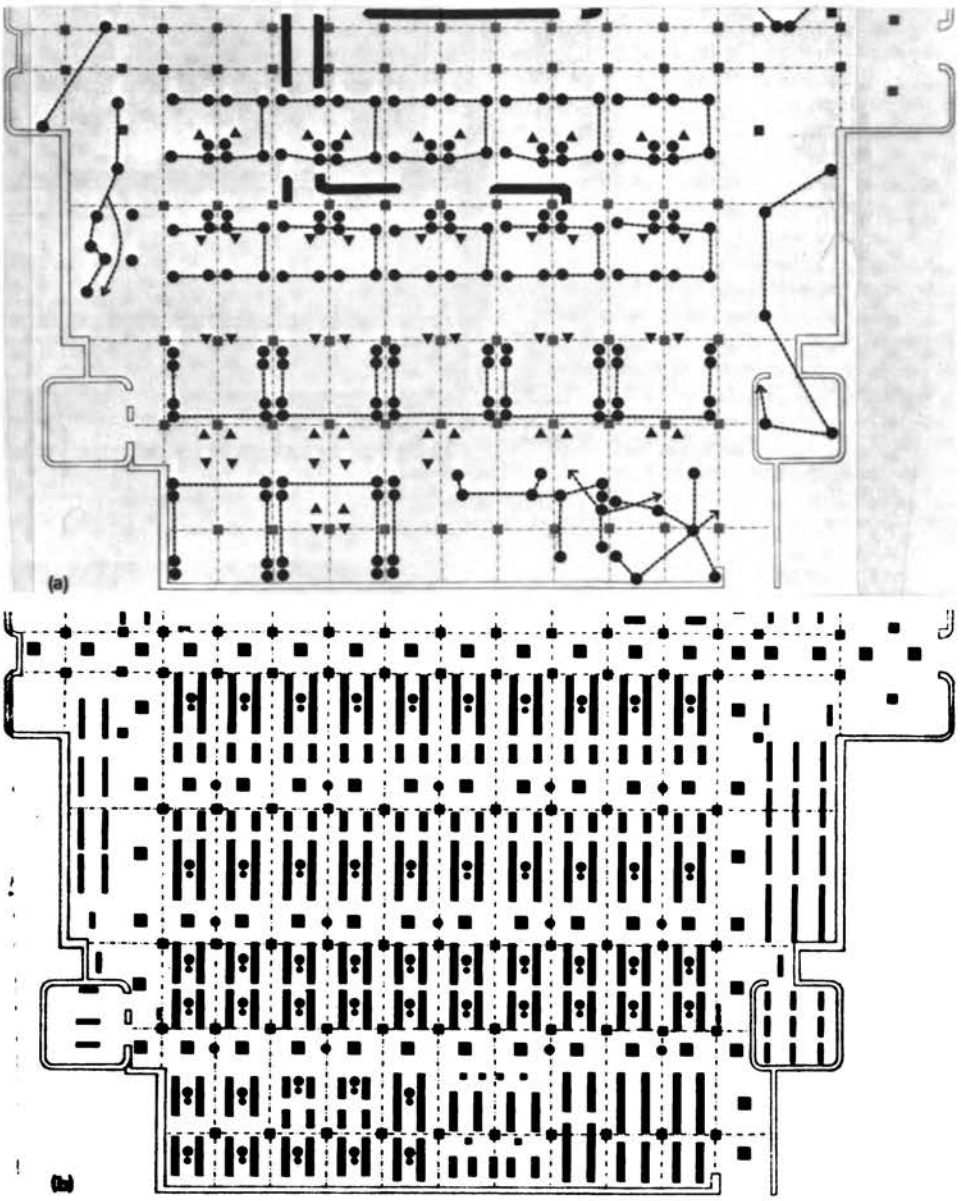

FIGURE 5 (a) Electrical power plan; (b) electrical lighting plan.

fully to the design of laboratory animal facilities in our society; furthermore, the idea of a "static" or "permanent" animal facility would be a delusion. Therefore, one can easily begin to appreciate the advantages of an important recent development in design: that many research environments should be seen as ever-changing, and rarely static. Correspondingly, few or no walls should be built to achieve an open-plan effect. Because of barrier and control concepts in laboratory animal housing, completely open plans are not acceptable, but the analogous field theory or open geometry of 3-dimensional modular planning, with corridors that can be placed anywhere, and with service utilities available at any point in the area, appears to hold great promise for the fourth dimension of planning, namely, the accommodation of time-based changes.

I have appeared to digress by tracking through the open country of open geometry and the narrow canyons of closed geometries. My intention is to provide a background of an architectural and engineering solution for *permissive* planning, that is, the development of an environment that is forgiving of management's changes of mind, technological obsolescence, errors in judgment, programmatic developments, and evolution of biohazard containment. The technology exists for architectural and engineering expressions of permissive planning, along with the professional expertise

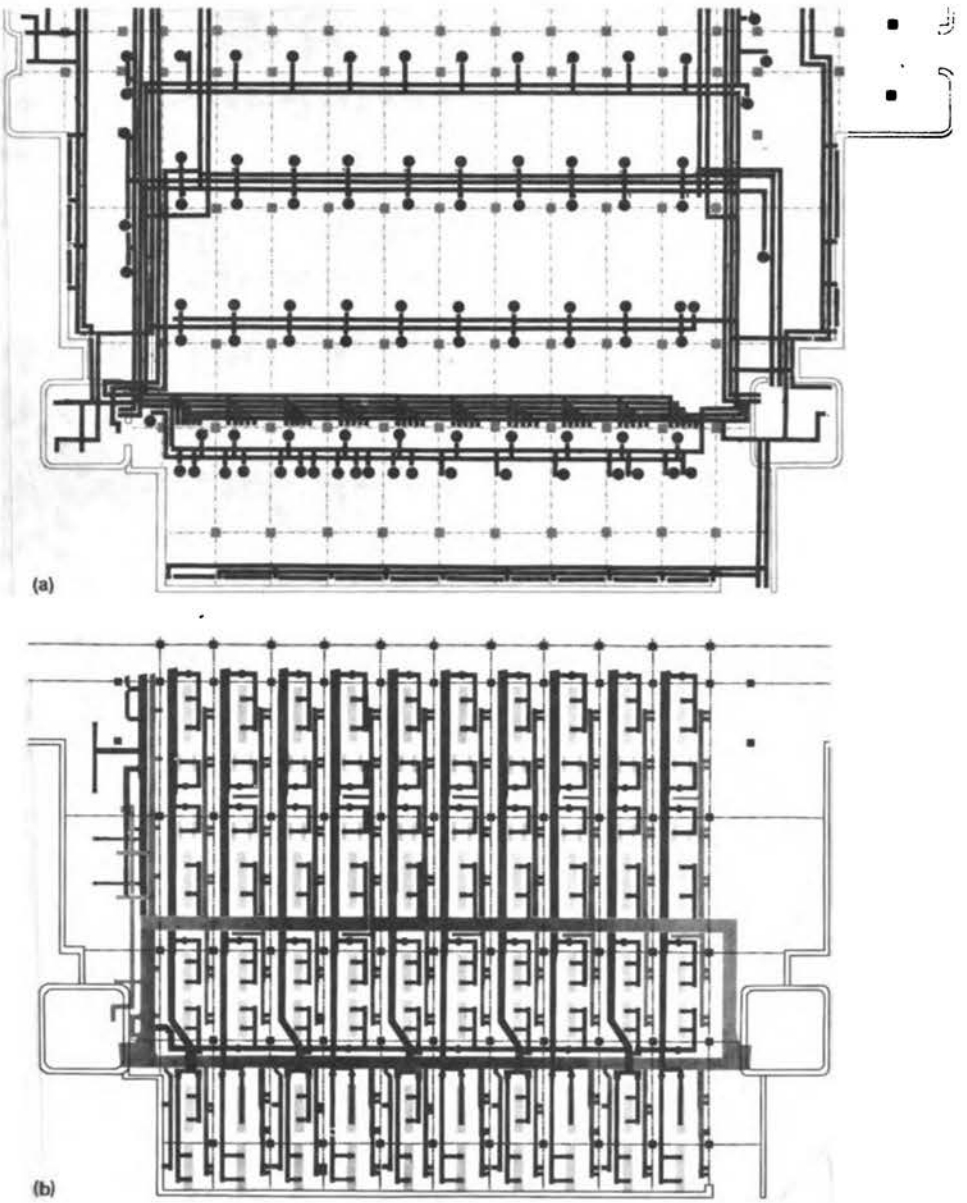

FIGURE 6 (a) HVAC piping plan; (b) HVAC ductwork plan.

to deliver the technology, and there is probably only a 0 to 10 percent initial investment premium for such flexibility (see Figure 7, for example).

Returning to the orchestration of information, what are management's emphases that become the determinants for arranging the information categories in hierarchies that, in turn, become translated into a physical design? Such emphases are those of *your* selection, if you are the facilities manager. For example, are you going to emphasize biohazard containment suites to the point of excluding simple animal rooms off of a single-loaded corridor? Or are you going to emphasize simple animal rooms off of a single-loaded corridor to the point of exclusion of biohazard containment suites? Or do you want to have the long-term option of any proportion of both? These answers will determine in the simplest and the most profound ways the organization of information, which in turn will influence the design of your facility.

These interrogations should be repeated through every aspect of the information, until relative emphases have been applied to all categories. Management itself must ultimately establish the relative primary, secondary, tertiary, etc., levels of emphasis; management must also establish the time-dependent elasticity in its designations, i.e., the extent to which emphases

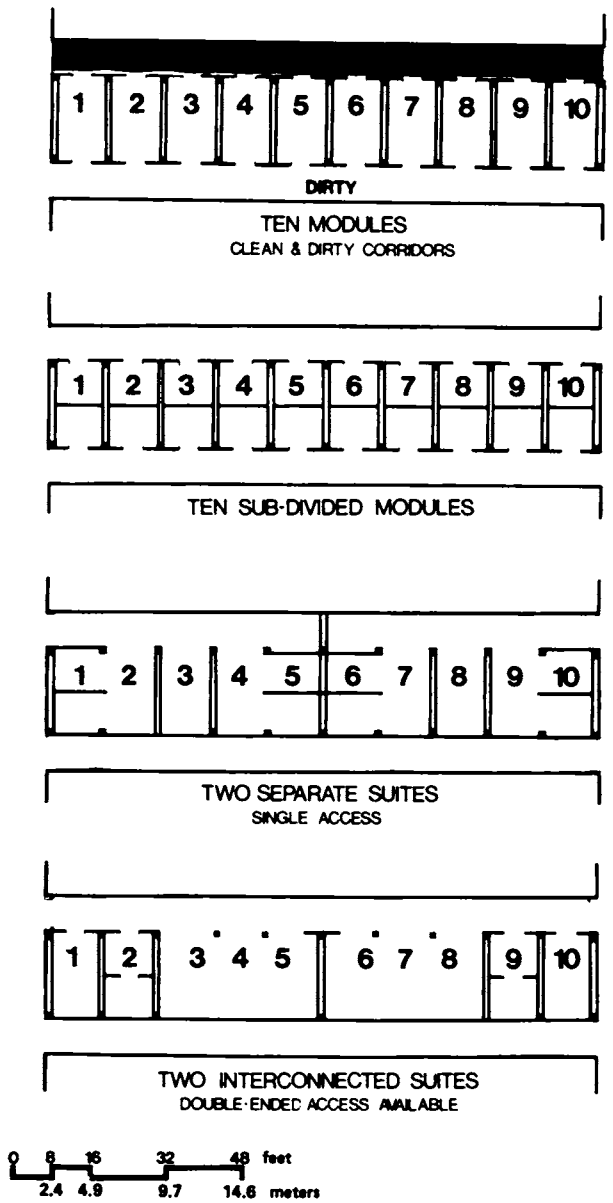

FIGURE 7 Tier 4—potential arrangements.

may change over time. Once the designer begins to make diagrams, ambiguities and conflicts will normally be revealed in the emphases previously designated, causing feedback and reevaluation of prior designations with a view to changing emphases by a consideration of trade-offs.

As an excellent enunciation of concepts of relative emphases and of diagrams, I suggest Christopher Alexander's book, *Notes on the Synthesis of Form* (1974 printing). In his preface to the paperback edition, Alexander argues:

> The idea of a diagram, or pattern, is very simple. It is an abstract pattern of physical relationships which resolves a small system of interacting and conflicting forces, and is independent of all other forces, and of all other possible diagrams. The idea that it is possible to create such abstract relationships one at a time, and to create designs which are whole by fusing these relationships--this amazingly simple idea is, for me, the most important discovery of the book.
>
> I have discovered, since, that these abstract diagrams not only allow you to create a single whole from them, by fusion, but also have other even more important powers. Because the diagrams are independent of one another, you can study them and improve them one at a time, so that their evolution can be gradual and cumulative. Most important still, because they are abstract and independent, you can use them to create not just one design, but an infinite variety of designs, all of them free combinations of the same set of patterns.
>
> As you can see, it is the independence of the diagrams which gives them these powers.... (p. i)

The phrase "independence of the diagrams" refers solely to the fine reductive detailing of Alexander's categories. Combinations of ideas, as with diagrams, lend interdependence, and the theme of interdependence of combinations of ideas must be stressed here. All pertinent ideas eventually become linked, directly and indirectly; thus trade-offs assume great importance in dealing with relative emphases.

Another noteworthy aspect of the linkage between design and management is what some have called the application of game theory--the comparison of planning strategies and tactics. Again, the bases of such comparisons are the felt needs of management--but the initial stimulation of such ideas only begins the architectural and engineering design team's complicated work of making space, time, engineering, and dollar comparisons.

Variations in size and scope are inherent among the differing types of animal facilities' sponsors--medical schools, government agencies, hospitals, breeding farms, corporate research organizations--and there will be differences among sponsors of the same type. In sum, it is highly unlikely that two animal facilities will ever be created alike. Therefore, we often perceive each building as a "one-of-a-kind" structure, a prototype never perfected, and never fully understood by its own builders and creators. But with an open-plan approach, an architect or engineer might draw nearer to a "perfect" design than any previously realized because of the relative ease of altering it.

Animal facilities' managers may prefer to create their own criteria (or emphases) for evaluating the built environment in which they operate. Yet common ground does exist, and national standards can be proposed for many architectural features, because analogous criteria for assessing such institutions as schools, hospitals, theaters, and nursing homes have been

developed. For animal facilities, evaluations should be made of space organization and allotment, building materials and finishes, heating, ventilating, lighting, and acoustic environmental systems, plumbing supply and waste systems, biohazard controls, energy conservation measures, materials handling, solid waste disposal, and automatic flushing systems. I have noticed many aspects of laboratory animal housing in which gaps can be discerned in the emphases applied by diverse managers of animal quarters. The most prominent differences of opinion center around the following features:

- the sizes of rooms for small animals;
- an optimal length or width of an animal room in any given setting;
- the number of rooms of any given size or range of sizes;
- the possibility of separating an operational area from a room housing small animals;
- the too-easy compromise that defeats the purpose of separating "clean" from "dirty" corridors;
- the use of large sinks in animal rooms, which might encourage dumping of cage bedding and from which aerosols would be released;
- the decision to equip (or not to equip) animal holding rooms with laboratory service utilities;
- the selection of major washing equipment components and systems; and
- the preference for rooms of fixed versus variable functions.

For further examples, it is instructive to read many of the other papers presented at this symposium, and then note the recurrent recognition given "institutional compromises" and constraints that weaken the good intentions of management. In general, those references appear to suggest differences in institutional values, as well as the lack of recognition of basic planning issues. Yet such common misperceptions serve to make my point--that gaps in understanding can skew emphases in information-handling. And, most assuredly, what a manager fails to emphasize to the designer will be blamed on the designer forever. I am not recommending a designer's unquestioning acceptance of every aspect of a particular manager's request. On the contrary, the competent design-construct team (consisting of an architect, an engineer, an estimator, and a construction manager) will not only logically challenge every aspect of the facilities manager's requests, but will present options and explain the architectural consequences for each alternative.

SUMMARY

To paraphrase Frank Lloyd Wright (1949), our architectural and engineering designs spin a most complex web of multiple systems, all resulting from the foregoing universe of concepts and information and all having to be considered by the entire team of design specialists and generalists. The modern laboratory animal facility is a complex series of systems (illustrated in Figures 1-7) based upon the coherent organization of information. Each proposed laboratory animal housing project--be it new or remodeled, centralized or satellite--should have a descriptive listing of information organized by category. The categories can be arranged into hierarchies determined by the management's value judgments, emphases, and informed trade-offs. The emphasis applied to the information will guide the designer's translation of the information into a workable design. The facilities manager must be prepared to collaborate with the design-construct team to achieve early delivery at lowest cost. Flexible open-geometry or field-theory planning concepts may offer fewer long-term hindrances than conventional linear systems to the effective planning of laboratory animal complexes. Values, emphases, and priorities, as well as conflicts, emerge from an institution's detailed definition of its goals for laboratory animal housing, all of which will change as time goes by. Thus, orchestrated descriptive information about the intended animal facilities will be the major determinant of the architectural plan that will result.

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Discussion

STEVENS: I am Christine Stevens of the Animal Welfare Institute. Mr. Goldstein did not elaborate very much on the use of space without permanent walls. I would like to know more about how the "open-plan" concept applies to laboratory animal housing.

GOLDSTEIN: The "open-plan" concept itself is not applicable for the most part. It could be adapted to animal housing if the building designer had the necessary systems, such as the electrical and plumbing systems, designed so that corridors and walls could be placed and replaced in the animal area without constraint.

CASS: I am Dr. Cass from the Veterans Administration, Washington, D.C. One of the questions that comes to mind as we talk construction is, "Are we talking simply about the warehousing of laboratory animals?" Laboratory animal science, as well as laboratory animal medicine, may be at the point where we should concern ourselves with the total study setting for animals. I wonder if, when we talk about construction, we are looking at too small a fraction of the complexities involved.

FOSTER: I am Henry Foster of Charles River Laboratories. My question may seem facetious, but I am very serious. If we all had unlimited budgets, is there enough known to build the perfect animal facility? For example, do we know what a perfect floor material is? What a perfect filtration system is? What perfect cage design and room size are? In other words, is money a limiting factor or is the technology still evolving?

MORELAND: I do not think our expertise has evolved to the point where we could produce the perfect animal facility. The continually evolving needs and requirements of a research program make an ideal facility impossible. A supposedly simple item like room size is an extremely difficult subject to generalize about. For some programs, large rooms are certainly much more desirable than small ones. Different room sizes are required for animal breeding facilities than for animal housing facilities. To cite another example, if the acquisition and quarantine programs are of high quality, and are provided or can be provided in one setting, the necessity of having numerous small rooms to prevent the spread of disease from one area to another is eliminated. But if a facility is designed with small quarantine rooms and the research program changes, those rooms would be too small for any other function.

SOURI: I am Elias Souri from Searle Laboratories. We are in the process of having an architectural firm design a building for us. It is a 3-story building and we have to decide how to deal with clean and dirty corridors, and the flow of animals and people. We have not yet decided what we are going to do, and I would like to know if there is an optimal pattern, not only in a 2-dimensional sense, but also in a 3-dimensional one?

JONAS: There is no simple answer to that question. I prefer multiple-corridor systems, because I think they lend themselves more easily to the efficient design of traffic flow patterns. You can compare multiple corridors to heavy city traffic, and the utility of 1-way traffic lanes is fairly well established. Logistically, we have a fair amount of experience with 1-corridor, 2-corridor, and 3-corridor systems, and with systems with

vertical stack arrangements (in which a basement is connected with a 3-corridor system). I think the people like to work in multiple-corridor systems better, too, because they can control their activity much better.

The next step is to go to a so-called barrier system and then integrate your multiple-corridor systems, decisions that revolve around the quality of animal coming into the facility, and not only the physical monitoring systems but the capability of the institution's personnel monitoring systems and biological monitoring systems.

Some difficulties that have been thought of in terms of the so-called barrier systems or clean-dirty corridor systems may usually be laid to deficiencies in management and quality control rather than to a basic flaw in the concept of a clean-dirty or an entry-exit system. These systems can be very flexible if they are well planned from their inception.

MORELAND: There must be an institutional commitment to the necessity and the value of a clean-dirty corridor system, which offers an extremely valuable way to contain infection. However, the institution has got to be committed to the efficient use of the corridors or else they are a waste of money. It is unlikely that a clean-dirty corridor system is going to be a total success in a medical school. I would compare it to the difficulties in designing a primate facility that would absolutely prevent escape of a primate. We have a small isolation facility for infectious diseases in our school, and we gave a great deal of thought to its design so as to prevent breakdown of the system. Yet the scientists at our institution are ingenious indeed. They have found ways to block the doors open, to bypass the showers, or to do whatever is necessary for their convenience, but they simply are not going to comply with the rules of the system. The system would probably work in an institution in which you have rigid control of the people who use that system, in which you can establish an orientation program for new employees to make sure that they understand the necessity and the desirability of the system and the reasons for using it properly, and in which stiff penalties could be administered for jimmying the system.

NEIL: I am David Neil of Colorado State University. My question concerns the matter of centralization. No matter what degree of centralization of animal care services exists in the physical sense in an institution, isn't the centralized management of personnel throughout the facility essential or even mandatory? Such control would contribute to uniform ethical and scientific standards and provide the institution with greater flexibility.

JONAS: Yes, centralized management is critical to the overall program. I would like to reinforce some of the points that you cited, but first I should say that there are many advantages to carefully considered dispersed facilities. In my own institution and in many I have visited, dispersion lends itself to good scientific productivity. Pressure comes from many sources, especially in institutions that have not experienced centralized management, to resist centralization and have the dispersed facilities managed independently. We must also realize that some of these very specialized facilities demand very specialized training in order to manage them. Therefore, if the managers and the professional team are going to take the responsibility of managing or interacting with these special facilities, then they must have the competence to do so. Investigators become rightly concerned if an unknown person comes in to take over their delicately maintained specialized genetic stocks or successful specialized programs. Investigators view their animal facilities as their research laboratories. If new management is introduced, competence should be demonstrated.

POVAR: I am Morris Povar from Brown University. We have listened to a great deal of discussion about design features and optimum utilization of space. We have not talked at all about keeping the system going. Having worked in a facility now since 1969 and having had great trouble keeping the fundamental systems operating from day to day--such as air turnover and ventilation in general--I wonder about the possibility of obtaining competence in the engineering field. Mr. Goldstein mentioned very quietly and then slipped over the difficulty in finding competent architects and designers and the total lack of backup systems. We have shutdowns of 3 days to a week when boilers, steam generators, or coils break--entire operations and research are disrupted, because no backup measures are built into these systems. This seems to be a common fact of life. The animals are likely to be subjected to stress when temperatures rise because we have no way of lowering the temperature if part of the air-conditioning system fails. This danger is inherent in small satellite facilities and central facilities. I would like to hear how other people cope with this, because we have failed miserably.

GOLDSTEIN: In the systems with which I have been associated over the last 13 years, we have been conscious of the basic necessity to have a backup system for all air handling for animal facilities. I am aware that very few of the 61 institutions that I have visited in this and 6 other countries have had these backup faciliites vis-à-vis air handling. It is commonplace, however, for emergency systems to exist. I am not sure whether you were referring to backup measures in an operational sense or only to emergency systems. Emergency boilers and chillers are rarely employed, but emergency generators enjoy a fairly wide usage. With the proliferation of sophisticated systems, we must come to rely upon an automatic data-

processing system to activate the backup for complex and sophisticated heating, ventilation, and air-conditioning control systems that will totally or partially fail from time to time. I think that the intricacies of our current systems usage demand more and more thoughtful approaches to backup. How that approach is conceived is a matter for ingenuity in engineering and management; of course, the amount of available money is crucial because a finite and significant investment of capital is needed to support backup systems and their controls.

MORELAND: A partial solution would be to obtain increased financial support from institutional administration. Competent maintenance personnel can repair any piece of equipment that we install today. Yet, the institutional administration, in an attempt to save a dollar, will often employ someone who is not sufficiently expert. If institutions are willing to spend money on highly skilled maintenance staff and on quick replacement of parts, then there is no reason why repairs cannot be speedy. It may be impossible to maintain a store of spare parts that would allow you to respond immediately to any emergency, but, if your spending procedures are not too constricted, the duration of these breakdowns and failures can be kept to a minimum. I would also remind you that, in the survey I took, the respondents did not state that any animals had died from even lengthy outages or failures of emergency systems. Although I did not directly ask if animal deaths had occurred, I believe that they would have told me had there been any.

JONAS: Dr. Povar has touched upon one of the major headaches in any facility--the effects of down time and the need for a practical backup system. For example, instead of having one major fan system for air handling, it might be safer to have two systems operating together to deliver the total. Therefore, even when one is down, it is possible to run the system at half capacity and still maintain a balance. Architects should be looking at such critical considerations, which, in the past, have not been accounted for in some designs. As you gain practical experience in a facility, you start finding out what its deficiencies are, and if you talk to other people and manufacturers of equipment, you find that certain items often need replacement. I am a firm believer in having a very good parts pool. We maintain our own centrifugal pumps, at $1,500 a pump, in stock. When new units are required, it takes about an hour to pull out the old pump and replace it with a new one. Because we could not tolerate prolonged down times, we have circumvented them accordingly.

DAVIS: My name is John Davis. I am an architect with the University of Texas. I am besieged with requests for flexible animal facilities, which we have talked about somewhat this morning. Yet, I also constantly hear requests for inflexible items such as seamless floors, walls, and ceilings; specially built mechanical systems; and air conditioning that requires 100 percent exhaust air, sometimes with laminar flow. Lighting and power requirements are also inflexible by their very nature. I can appreciate what Mr. Goldstein has said about open planning, as we have attempted to build this type of facility within the University of Texas system. Yet when we return only months or years later to make these easy, inexpensive remodelings, we find ourselves paying more for the renovation than we paid for the original installation. I am not sure that we have fully resolved the question of flexibility.

GOLDSTEIN: I was not attempting to suggest that change came easily or that it took place without a price. Considering the current rates of economic inflation in the construction industry, the costs of any change are probably going to exceed the original cost by itself: replicating a room, for example. However, if a building concept is not reasonably permissive of change, the whole building can become obsolete. Although we have learned to adapt almost anything to our purposes, it is a fact of life that buildings do get abandoned. It was in this context that I suggested an approach--an engineering, managerial, and architectural concept--that might be more permissive of change.

GREENSTEIN: I am Ed Greenstein of the National Institutes of Health. I would like to suggest that preventive maintenance and a modular system of equipment design, in which parts that tend to break could easily be plucked out and new panels inserted, would reduce down time tremendously. I do not know if any such units exist, but certainly they should be planned and designed. Preventive maintenance should mean that we follow the transportation industry's example by checking our equipment regularly, and having our local engineers inspect facilities and try to correct failures before they happen. I think that inspections would reduce down time and catastrophe time.

II

The Animal Environment

Physical, Chemical, and Microbial Factors Affecting Biologic Response

J. RUSSELL LINDSEY, MICHAEL W. CONNER, and HENRY J. BAKER

A large proportion of the world's biomedical scientists are primarily concerned with measuring biological responses of animals. The integrity of their research, regardless of discipline, frequently is influenced by their concept of the animal. For this reason, we will first present a conceptual view* of the modern laboratory animal and then utilize the remainder of the paper to provide documentary evidence in support of its validity.

CONCEPTUAL VIEW OF THE MODERN LABORATORY ANIMAL

Although commonly viewed as such in the present-day research system, laboratory animals are not standard commodities to be purchased and stored like soap or toothpaste. Furthermore, they never will be because of the sheer complexity and number of delicately balanced biological systems they represent. This is not to say that animals cannot be standardized to a substantial degree. The problem is that the *degree* of standardization may vary enormously, even within those strains having the greatest genetic uniformity.

How then should the laboratory animal be viewed in the context of today's sophisticated science? We recommend the following. *In terms of biologic response(s), every experimental animal is a composite of genetic and environmental effects--at each point in time from zygote to ultimate death* (Figure 1). In other words, the biologic response is merely an expression of *both* genetic and environmental effects. The concept of genetic influences on biologic response is widely accepted; only recently have we come to realize that environmental influences often have profound effects on biologic response. In addition, one should consider that the real-life situation is probably further confounded by complex interactions between genetic and environmental influences. Quality control has the important role of maintaining genetic purity while preventing, minimizing, or maintaining as nearly constant as possible the effects of various environmental factors (ILAR, 1976).

The purpose of this paper is to bring together much of the data concerned with environmental factors (physical, chemical, and microbial) affecting biologic responses of the more common laboratory animals. Dietary factors (Newberne and Fox, 1978) and factors affecting behaviorial responses (Davis, 1978) are treated elsewhere in this volume. Some genetic and environmental factors affecting biologic responses were the subject of a recent symposium (Lang and Vesell, 1976).

PHYSICAL FACTORS

Temperature and Humidity

Only in recent years has there been much effort to define the animal's microenvironment (the cage) in terms of temperature and humidity and to relate these conditions to those of the macroenvironment (the room) (Institute of Environmental Research, 1971). Complex interactions exist between production of heat and water inside the cage and their dissipation into the macroenvironment (Woods, 1978). These interactions are known to be influenced by many factors, including size of animal population (Yamauchi *et al.*, 1965), cage design (Serrano, 1971), presence or absence of

*The concept to be presented is concerned exclusively with biologic response, not man's total concept of living animals.

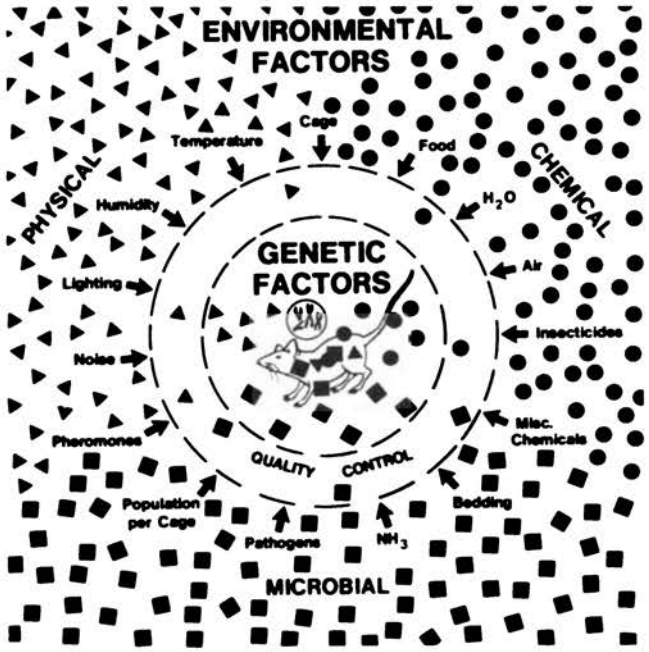

FIGURE 1 Conceptual view of the modern laboratory animal. *In terms of biologic response, every experimental animal is a composite of genetic and environmental effects–at each point in time from zygote to ultimate death.* Many physical (▲), chemical (●), and microbial (■) factors of the environment contribute along with genetic factors (of the genome), toward each animal's responsiveness to experimental stimuli. Quality control has the important role of maintaining genetic purity while preventing, minimizing, or maintaining as nearly constant as possible the effects of various environmental factors.

filter tops (Simmons *et al.*, 1968; Besch, 1975), and the amount and velocity of air flowing over the cage (Woods, 1975). These parameters ultimately determine the temperature of the animal's microenvironment, which is generally a few degrees in temperature and a few percentage points in relative humidity (RH) above that of the macroenvironment. Unfortunately, there are few studies (compared to studies involving cold and heat stress) relating the relatively small increases in temperature and humidity of intracage conditions to actual effects on biologic responses.

One of the most obvious indicators that temperature and humidity can seriously affect laboratory animals is the disease known as "ringtail." This disease, characterized by annular constrictions around part or all of the tail, with or without subsequent sloughing, almost certainly bespeaks dramatic physiological changes. The precise mechanism responsible is unknown, but it is generally thought to be associated with inability of the very young animal to control heat loss in an environment of low (below 40 percent) RH (Njaa *et al.*, 1957; Totton, 1958; Flynn, 1959; Stuhlman and Wagner, 1971). Ringtail has been observed most frequently in rats, but mice (Nelson, 1960) and the South African hamster, *Mystromys albicaudatus* (Stuhlman and Wagner, 1971), also can develop the disease.

Temperature and humidity are important parameters of the thermal environment, as together with air movement they are critical determinants of heat loss or retention by convection and radiation and, thus, metabolic rate. In general, temperature and humidity levels for laboratory animal environments are selected to at least roughly coincide with the "thermoneutral zone" (that temperature and humidity at which heat is neither gained nor lost) for each animal species. This is approximated by the current recommendations of 21.1-26.7°C for rats and mice and 21.1-23.3°C for guinea pigs and hamsters (with fluctuations not to exceed ± 1°C) at 40-70 percent RH (ILAR, 1969).

Weihe (1973) recently has reviewed the effects of temperature on drug action. He emphasizes the importance of adequately defining the size of animal populations, types of cages (wire mesh or boxes with bedding), and the ambient temperatures used in drug toxicity trials. An experiment is cited in which the LD_{50} for amphetamine at 27°C averaged 78.9 mg/kg for singly caged mice compared to 11.6 mg/kg for mice housed in groups of 10. This sevenfold difference is apparently due to the higher body temperature of the group-housed mice. Group-housed mice tend to huddle together, particularly when the temperature falls below the thermoneutral zone.

Weihe (1973) emphasizes the existence of at least two patterns of drug toxicity responses (first described by Fuhrman and Fuhrman, 1961) dependent on cage temperature in mice and rats. The first pattern is a V- or U-shaped curve, with minimum toxicity around thermal neutrality and increasing toxicity at lower and higher cage temperatures. The second pattern is linear; toxicity is directly correlated with increasing cage temperature.

In studies of drugs affecting homeothermia, animals kept at a room temperature corresponding to their thermal neutrality may be unsuitable. For example, interference with homeothermia by blockage of adrenergic functions or stimulation of cholinergic functions can only be studied in cold-exposed animals (Weihe, 1973).

Baetjer (1968) reported that temperature and humidity can influence susceptibility to infectious diseases. She found that chicks exposed to a standard dose of Newcastle disease virus were more susceptible at 28.9°C, 20 percent RH, than at 28.9°C, 90 percent RH, and more susceptible at 22.2°C, 90 percent RH than at 28.9°C, 90 percent RH. She also reported that mice were more susceptible to the PR8 strain of influenza when maintained at 35.6°C, 22 percent RH, than at 35.6°C, 90 percent RH.

Lighting

Multiple Effects of Light Animal facility lighting is known to have important effects on biologic responses, but has not been studied extensively. The photoperiod, the number of hours of light per 24-hour day, is generally accepted as having great influence on reproduction; long days (13-14 hours) produce the best results (Mulder, 1971). Photoperiodicity

regulates, but doesn't control, circadian rhythms (Hastings and Menaker, 1976; Palmer, 1976).

The effects of light intensity (measured in footcandles as lumen per square foot or lux as lumen per square meter) and quality (wavelength spectrum as Angstrom units, Å) are less well understood in terms of the levels most beneficial for, or their effects on, laboratory animals. The current recommendation is for 25 footcandles at floor level (ILAR, 1972). However, intensities of 100 footcandles (O'Steen and Anderson, 1972) and lower (Weihe *et al.*, 1974) are known to cause degeneration of photoreceptors in the eyes of rats. The effect becomes more pronounced with increasing age (O'Steen *et al.*, 1974; Weihe *et al.*, 1974).

Circadian Rhythms in Biologic Responses The term "circadian rhythm" (meaning "around a day"), introduced by Halberg *et al.* (1959), refers to endogenous rhythms or "biological clocks" of physiologic functions in man and animals (Hastings, 1970). Until recent years, it has not been fully appreciated that these rhythms may profoundly influence experimental data. Illustrative examples are shown in Figure 2, which is concerned with three different biologic responses: duration of narcosis after pentobarbital, time to death after whole-body irradiation, and mortality from a standard dose of ethanol. It should be noted that the parameter least affected, pentobarbital sleep time, deviated from the 24-hour mean by a total of approximately 50 percent during a full 24-hour cycle (Aschoff, 1967)!

The number of behavioral, physiological, and biochemical parameters known to have a 24-hour periodicity is impressive indeed. A partial listing is presented in Table 1. For more complete lists, the reader is referred to a number of recent reviews and books on this subject (Folk, 1966; Sanvordeker and Lambert, 1974; Palmer, 1976; Romero, 1976). The controlling mechanisms are both complex and variable, depending on the biologic response to be measured; but, in general, information about environmental lighting indicates that light is converted into neural signals by specialized photoreceptors in the eyes, processed in the central nervous system where the signals can be translated directly into behavioral, physiological, and biochemical phenomena, or first modulated by a variety of neuroendrocrine regulatory mechanisms (Hastings and Menaker, 1976; Romero, 1976).

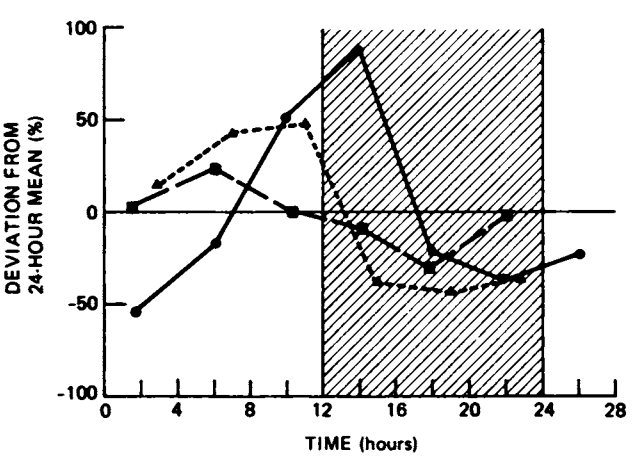

FIGURE 2 Rhythms in biologic responses of mice with photoperiod of 12 hours of light alternating with 12 hours of darkness (the shaded area indicates darkness). Open circles represent duration of narcosis after pentobarbital; the crosses, time to death after whole-body irradiation; the solid circles, mortality from a dose of ethanol. (From Aschoff, 1967.)

Noise

Data on the effects of noise on laboratory animals are few. Anthony (1962) has expressed the belief that rodents, like man, experience mechanical damage to the ears due to sounds of 160 decibels (db), pain at 140 db, and signs of inner ear disturbance after prolonged exposure to sounds around 100 db. Accordingly, he has recommended that permissable noise levels in animal facilities not exceed 85 db.

Geber *et al.* (1966) exposed rats to average sound levels of 83 db (octave band noise level 46-78 db for 20-4,800 cycles/second) for periods ranging from a few minutes to 6 minutes/hour for 3 weeks. Increases were seen in the number of eosinophils in the peripheral blood, serum cholesterol, adrenal ascorbic acid levels, and adrenal weights. Friedman *et al.* (1967) also have shown that noise (continuous sound and intensity of 102 db or intermittent sound of 200-cycle square wave with a duration of 1 second and an intensity of 114 db) resulted in elevations of serum lipids of rats on normal diets or rabbits fed cholesterol.

Isolation Versus Crowding

Biomedical investigators traditionally have housed laboratory animals in groups of uniform size, but usually without realizing the implications of their selected population densities on the physiologic responses under study. Similarly, more or less arbitrary space requirements for caging of animals have been established without objective data as to what population sizes best provide for the "normal" physiologic state (ILAR, 1972). In actual practice, such an ideal may not exist, but a large number of studies do provide data supporting the thesis that population can have considerable impact on biologic responses.

It has been known for many years that crowding of animals has serious consequences on reproduction (Christian and Le Munyan, 1958) and on behavioral responses such as fighting (Welch and Welch, 1969; Davis, 1978). However, it appears much less well appreciated that animal population density also has important metabolic effects.

Barrett and Stockham (1963) have shown that rats housed singly for 18 hours had plasma corti-

TABLE 1 Partial List of Biologic Responses Significantly Altered During the Circadian Cycle

Process or Event	Authors
Hepatic drug metabolism	Radzialowski and Bousquet, 1967, 1968; Nair and Casper, 1969; Jori *et al.*, 1971; Chedid and Nair, 1972
Pentobarbital or hexabarbital sleep time	Scheving *et al.*, 1968; Nair and Casper, 1969
Hepatic protein synthesis	LeBouton and Handler, 1971; Mitropoulos *et al.*, 1972
Drug effectiveness and toxicity	Haus and Halberg, 1959; Ede, 1974; Romero, 1976
DNA synthesis and mitotic activity	Nash, 1971; Izquierdo and Gibbs, 1972
Susceptibility of experimental leukemia to chemotherapy	Haus *et al.*, 1972; Halberg *et al.*, 1973
Serum cortisone levels	Alder and Friedman, 1968; Bowman *et al.*, 1970 Ramaley, 1972
Serum lipid levels	Cayen, 1972
Body temperature	Fioretti *et al.*, 1974
Susceptibility to infection	Feigin *et al.*, 1969; Wongwiwat *et al.*, 1972; Shackelford and Feigin, 1973
Susceptibility of rats to gastric erosions	Alder, 1967
Consumption of food and water	Murakami and Watanobe, 1973; Horton *et al.*, 1975

sone levels about half of those of animals housed in identical cages for the same time in groups of 20. They also have demonstrated that many nonspecific stimuli, such as change in environment, noise, handling, and giving injections, produce marked increases in cortisone levels lasting about 2 hours. Whereas, short-term (a few days) individual housing of rats results in lowered plasma cortisone levels, prolonged individual housing causes the opposite effect. Rats housed individually for periods of several weeks become irritable and aggressive, have larger adrenal and thyroid glands, and show increased hepatic microsomal enzyme activity. This is referred to as the *isolation stress phenomenon* (Dairman and Balazs, 1970). This type of stress has been shown to enhance ethanol consumption in the rat (Parker and Radow, 1974). Isolation stress apparently occurs in mice also (Consolo *et al.*, 1965).

Animal housing density also appears to have an important influence on a variety of immune responses. A recent paper by Joasoo and McKenzie (1976) is an excellent example. These researchers, using rats housed 1, 2, and 10 per cage and immunized with thyroglobulin, have shown that the *in vitro* response of sensitized splenic lymphocytes to thyroglobulin is increased by crowding and decreased by isolation of female rats (Table 2). Both crowded and isolated male rats respond by a decrease in reactivity of lymphocytes to the antigen. The responsiveness of sensitized cells is decreased by giving an injection of epinephrine 30 minutes before the rats are killed to harvest the spleen cells. Crowding also has been found to increase the resistance of female mice, but to decrease the resistance of male mice to tuberculosis (MacManus *et al.*, 1971). In other studies, crowding has been shown to increase susceptibility of mice to a coxsackie virus infection (Johnson *et al.*, 1963); increase susceptibility to experimental malaria, *Plasmodium berghei*, in mice (Plaut *et al.*, 1969); reduce antibody synthesis in mice (Vessey, 1964; Solomon, 1969); depress

TABLE 2 Effect of Isolation and Overcrowding of Immunized Rats on the *In Vitro* Incorporation of ^{3}H-thymidine by Antigen-Stimulated Spleen Lymphocytes[a]

Experiment	Sex	Thyroglobulin, μg	Mean (Percent) Response ±SE of (*n*) Cultures[b] for Rats Housed in Numbers per Cage of: 1	2	10
I	f	200	228 ± 19 (28)[c]	292 ± 17 (32)	360 ± 14 (75)[c]
		20	175 ± 14 (28)[c]	218 ± 14 (32)	269 ± 10 (76)[c]
		2	155 ± 8 (27)[c]	231 ± 18 (32)	251 ± 9 (68)
II	f	200	252 ± 19 (32)	265 ± 31 (28)	364 ± 28 (34)[c]
III	m	200	271 ± 17 (32)[c]	363 ± 31 (32)	275 ± 23 (28)[c]

[a]From Joasoo and McKenzie (1976).
[b]Data are shown as percent responses to the addition of thyroglobulin, compared with results in cultures to which no antigen was added.
[c]$p < 0.05$ for significance of difference of means as compared with data obtained from cells of rats housed two per cage (center column).

homograft rejection in mice (Rasmussen, 1969); and enhance development of autoimmune disease in rats (Amkraut *et al.*, 1971). It appears that environmental influences on activity of pituitary (Gisler *et al.*, 1971; Gisler and Schenkel-Hulliger, 1971), adrenal (MacManus *et al.*, 1971; Joasoo and McKenzie, 1976), and sex hormones (Cohn and Hamilton, 1976) contribute greatly to immune responsiveness of the individual.

CHEMICAL FACTORS

The number of environmental chemicals to which laboratory animals (and personnel) may be exposed is exceedingly large (Lang and Vesell, 1976; Newberne and Fox, 1978). In the following sections, attention will be focused primarily in three areas: chemicals affecting hepatic microsomal enzyme activity, chemical factors affecting immune responses, and gaseous pollutants derived from animal wastes.

Chemicals Affecting Hepatic Microsomal Enzyme Activity

Although similar enzyme systems are found in other organs such as kidney and lung, the majority of enzymes concerned with degradation of exogenous

TABLE 3 A Partial List of Variables Affecting Drug Disposition in Experimental Animals[a]

Variables in the External Environment	Variables in the Internal Environment
Air exchange and composition	Adjuvant arthritis
Barometric pressure	Age
Cage design materials	Alloxan diabetes
(crowding or exercise)	Cardiovascular function
Cedar and other softwood bedding	Castration and hormone replacement
Cleanliness	Circadian and seasonal variations
Coprophagia	Dehydration
Diet (food and water)	Disease
Gravity	hepatic, renal, malignant, endocrine (thyroid, adrenal)
Hepatic microsomal enzyme	Estrous cycle
induction or inhibition by	Fever
insecticides, piperonyl butoxide,	Gastrointestinal function, patency, and flora
heavy metals, detergents,	Genetic constitution (strain and species differences)
organic solvents, vinyl chloride,	Hepatic blood flow
aerosols containing eucalyptol, etc.	Infection
Handling	Malnutrition, starvation
Humidity	Pregnancy
Light cycle	Sexual activity
Noise level	Shock (hemorrhagic or endotoxic)
Temperature	Stress

[a]Modified from Vesell *et al.* (1976).

drugs and chemicals are found in the liver. Since they reside in the microsomal fraction, they are collectively referred to as "hepatic microsomal enzymes" (HME). Because of their key role in metabolism of known and potential therapeutic agents, as well as various toxic chemicals, they have received much attention in recent years. Much of the current knowledge on the subject was reviewed in a recent symposium (Lang and Vesell, 1976).

As pointed out quite ably by Vesell *et al.* (1976), even under the best of environmental conditions the HME of rodents are subject to wide fluctuations in activity from one group of animals to the next or from day to day. Thus, it is always imperative that generous numbers of control animals be used, even though all environmental conditions for the animals and all chemical procedures involved in the enzyme assays are carried out with the greatest precision possible. The HME are *extremely* sensitive to a great diversity of environmental chemicals that may enter animal facilities for one reason or another. In short, the HME are influenced by practically any environmental variable (Table 3).

The most notorious chemicals affecting the HME, aside from certain dietary contaminants (Newberne, 1975; Newberne and Fox, 1978), are highly volatile substances such as insecticides (Conney and Burns, 1972), various constituents of room deodorizers (Jori *et al.*, 1969; Cinti *et al.*, 1976), and several types of animal bedding containing aromatic hydrocarbons. Because of their highly volatile nature, sufficient vapors of some substances may drift into animal rooms from corridors and storage areas to alter HME activity. The best rule is to keep unnecessary chemicals out of the animal facility and to carefully screen and control those which must enter.

Animal bedding can have profound effects on metabolism of laboratory animals. Red cedar (Pick and Little, 1965; Ferguson, 1966; Vesell *et al.*, 1976) and pine (Vesell, 1967) are known to increase HME activity. Cedrene and cedrol, two volatile hydrocarbons in cedar and cedarwood oil, also increase HME activity (Wade *et al.*, 1968; Hashimoto *et al.*, 1972). Ground corncobs are sometimes contaminated by aflatoxins (Port and Kaltenbach, 1969). Vermiculite bedding may cause dehydration in young animals (Hastings, 1967). Vesell *et al.* (1973) have shown that dirty bedding significantly alters HME activity, but the active component(s) have not been identified.

Bedding is one of the environmental materials to which laboratory animals have most intimate exposure, particularly if contact bedding is used. One wonders whether it does not have many additional effects on biologic response, perhaps related to microbial contamination, dust content, and chemical content. This possibility is strengthened by the suggestion that certain woods contain constituents that may be carcinogenic (Acheson *et al.*, 1968; Schoental, 1973).

Chemicals Affecting Immune Response

There is no doubt that many environmental chemicals have important influences on host defenses, several of which are extremely subtle. In some instances, these subtle interactions with other environmental variables may be of major importance to proper interpretation of research data (Exon *et al.*, 1975). In other instances, they may go completely unnoticed.

A number of insecticides have been shown to alter immune response (Wasserman *et al.*, 1969) and some have been found to induce lymphocytopenia in mice (Keast and Coales, 1967). Lead, in subclinical doses, has been found to suppress resistance of mice to *Salmonella typhimurium* (Hemphill *et al.*, 1971), increase susceptibility of rats (Selye *et al.*, 1966) and chicks (Truscott, 1970) to bacterial endotoxin, decrease phagocytosis in rats (Trejo *et al.*, 1972), and decrease antibody formation in mice (Koller and Kovacic, 1974). Cadmium presumably produces similar effects (Schroeder *et al.*, 1965; Koller, 1973; Exon *et al.*, 1975).

Gaseous Pollutants from Animal Wastes

Scope and Status of the Problem Standard practice is to maintain laboratory animals in small enclosures that more (as with filter tops) or less (standard rod-type lids on box cages or open-wire cages) limit the free exchange of gases between the micro- and macroenvironments. Even in the face of an excellent supply of pollutant-free air in the macroenvironment, modern cage design generally allows some degree of accumulation of gaseous pollutants in the animal's immediate environment. This accumulation depends upon many factors, but especially the number of animals, type and amount of bedding, frequency and method of sanitization, many aspects of cage design, and air dynamics within the room (Woods, 1978). At the present time, the major recognized gaseous pollutants derived from accumulation of animal wastes within the cage are carbon dioxide and ammonia. Serrano (1971) has shown that, depending upon cage design and animal activity, the intracage level of CO_2 in standard mouse cages housing 8 adults may increase as much as eight-fold over room air (as high as 4,517 ppm). It is not known whether differences of this magnitude have biologic effects on mice.

Serrano (1971) also studied the levels of NH_3 occurring in mouse cages. He showed that NH_3 usually was not detected until the third to sixth day after changing, depending on population, but by the seventh day the NH_3 levels in cages housing 8 adults were 21 to 177 ppm, depending on cage design. Levels of 200 to 350 ppm were obtained when 16 mice were housed in each cage for 7 days without changing the bedding. Similar results were obtained by Murakami (1971), who also reported much higher levels at night and in cages housing males. Gamble and Clough (1976) showed that levels of NH_3 of 25 ppm and greater were exceedingly common in rodent cages. Also, they showed that the buildup of NH_3 was directly related to room RH. Flynn (1968) reported levels of NH_3 exceeding 700 ppm in mouse cages with filter covers of a special design.

The biologic effects of increased intracage NH_3 are poorly understood. Vesell *et al.* (1973) have shown that dirty bedding impairs hepatic microsomal enzyme activity in rats and suggest (Vesell *et al.*, 1976) that NH_3 is the pollutant responsible. While this is a most appealing hypothesis, further work will be necessary to rule out the possible influences of other factors in the dirty environment. Visek (1974) believes that as a metabolite NH_3 has, among other deleterious effects, severe effects on intermediary metabolism and shortens the life span of animal cells.

Extensive work with poultry implicates environmental NH_3 as a cause of keratoconjunctivitis (Carnaghan, 1958), poor weight gain (Charles and Payne, 1966; Kling and Quarles, 1974), and increased susceptibility to respiratory infections at NH_3 levels as low as 20 ppm (Anderson *et al.*, 1964, 1968; Andersen, 1970; Sato *et al.*, 1973; Kling and Quarles, 1974). For man, the threshold limit value (maximum allowable level for work environment of 8 hours per day, 5 days per week) is 25 ppm (American Conference of Governmental Industrial Hygienists, 1976).

Broderson *et al.* (1976), in a large number of studies, tested the hypothesis that NH_3, at levels normally encountered in rat cages, plays a contributing role in the pathogenesis of murine respiratory mycoplasmosis (MRM) due to *Mycoplasma pulmonis*. Pathogen-free rats were infected intranasally with a standard dose of the organism and maintained for 4 or 6 weeks in environments with NH_3 of known concentration (the lowest being 25 ppm) from natural or artificial sources. All levels of NH_3 consistently increased the severity of the rhinitis, otitis media, tracheitis, and pneumonia (including bronchiectasis) characteristic of MRM. Prevalence of pneumonia showed a strong tendency to increase directly with NH_3 concentration. The authors concluded that environmental NH_3, at concentrations commonly encountered in present-day cage environments for rats, plays an important role in pathogenesis of MRM.

We have confirmed in our laboratories (J. R. Lindsey and M. W. Conner, unpublished observations) that there is a direct correlation between the concentration of NH_3 in the cage and the development of lung lesions in rats infected with *M. pulmonis*. Parenthetically, it should be mentioned that the "abnormal respiratory histology" of Donnelly *et al.* (1974), which was later attributed to environmental NH by Gamble and Clough (1976, Figure 9), is actually typical of MRM due to *M. pulmonis* (Lindsey *et al.*, 1971). Broderson *et al.* (1976) have shown that exposure of known pathogen-free rats to NH_3 alone results in morphologic changes only in the nasal passages.

MICROBIAL FACTORS

Microbial flora, both normal and abnormal, constitute a most important part of any animal's environment, particularly in regard to biologic response. Only selected aspects can be presented here, as the subject greatly exceeds the scope of the present paper.

Intestinal Flora

Under normal circumstances, the intestinal tract of the newborn mouse is colonized by different bacteria in a regular sequential manner (Dubos *et al.*, 1965; Schaedler *et al.*, 1965). Alterations in this normal flora can influence a variety of biologic responses, including susceptibility to bacterial endotoxin (Schaedler and Dubos, 1962), growth rate and infection (Dubos and Schaedler, 1960) and response to numerous chemicals (Williams, 1972), and can result in lasting physical and chemical effects (Dubos, 1969; Lee, 1970; Lee and Dubos, 1972a,b).

Flora Affecting Response to Radiation

A large variety of bacteria, the most notable being *Pseudomonas aeruginosa* (Flynn, 1963a), have been shown to dramatically alter the response of mice to whole body X-irradiation (Stoner *et al.*, 1965; Fritz *et al.*, 1968). Animals receiving a lethal dose of X-irradiation die much earlier if they have been infected with *P. aeruginosa* than if they have not been infected, as shown in Figure 3 (Flynn, 1963b). Similar effects have been reported for mice latently infected with *Hexamita muris* (Meshorer, 1969).

Effects of Latent Pathogens

A large number of other agents, predominantly latent pathogens of laboratory rats and mice, have been demonstrated to significantly affect experimental data under certain circumstances. The enormity of this problem can be appreciated only as one considers the fact that most conventional colonies maintain indigenous infections of latent pathogens (Parker *et al.*, 1966). *Mycoplasma pulmonis* is one of the most common microorganisms that infect mice and rats, particularly the latter. Because of the usual chronicity and slow cumulative mortality of the disease it produces in rats, it has had an enormous impact on longevity and possibly other parameters in many long-term studies (Lindsey *et al.*, 1971). Additionally, *M. pulmonis* has been observed to increase the frequency of lung cancers following administration of a respiratory carcinogen (Schreiber *et al.*, 1972) and to alter mucus secretions and mucociliary function in latently infected rats (Ventura and Domaradzki, 1967; Green, 1970; Irvani and van As, 1972). Infection by *Mycoplasma arthritidis* is known to increase susceptibility of rats to experimental pyelonephritis due to *Escherichia coli* (Thomsen and Rosendal, 1974) and to suppress humoral (Kaklamanis and Pavlatos, 1972; Berquist *et al.*, 1974) and cellular immunity (Eckner *et al.*, 1974). Simberkoff *et al.* (1969) have shown that in tissue culture *Mycoplasma* extracts can inhibit lymphocyte mitosis and antibody formation.

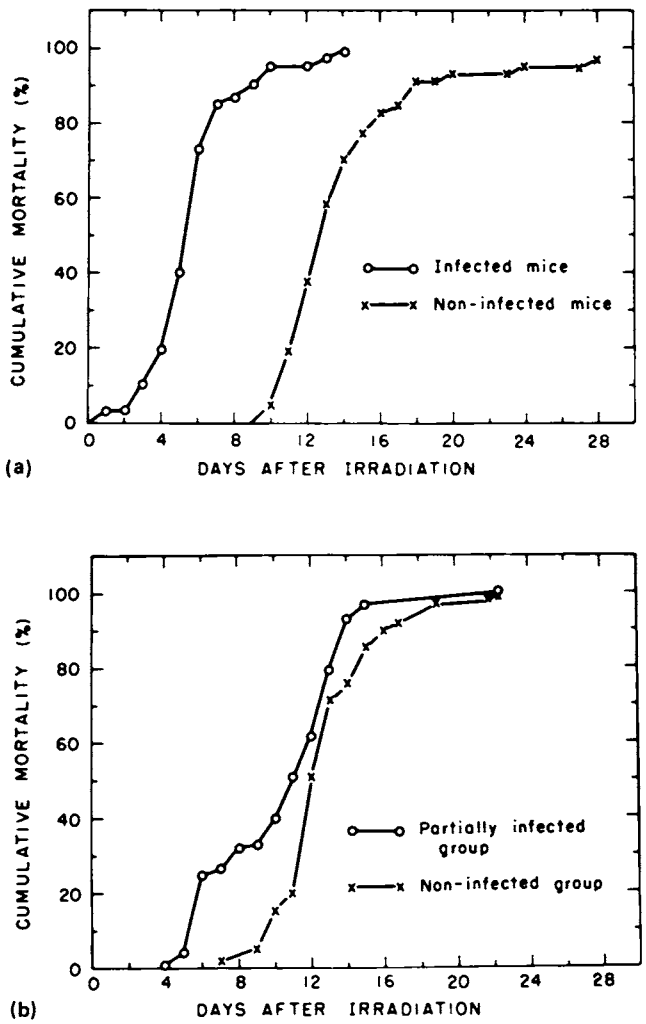

FIGURE 3 Typical survival curves of mice following lethal X-irradiation: (a) when all mice are either infected with *Pseudomonas aeruginosa* or free of this infection, and (b) when only some of the mice are infected with the organism. Many other latent infections, mostly gram-negative rods, have been incriminated in similar erratic responses. (From Flynn, 1963b.)

The lactic dehydrogenase virus (LDV) of mice, called by Riley (1974) the "benign modifier of body chemistry," is one of the most striking examples of an infection that can influence experimental results. It has been a common contaminant of tissue cultures and transplantable tumors of mice in the past. Infection of mice, usually by injection of contaminated materials passaged in mice, results in entirely silent clinical infections with profound consequences on many systemic functions. A partial list is given in Table 4.

The rickettsial agents, *Hemobartonella muris* of rats and *Eperythrozoon coccoides* of mice, have been implicated in altering host responses to numerous experimental infections and investigations involving phagocytic capacity of the reticuloendothelial system (Baker *et al.*, 1971). These are normally latent infections in which a few organisms persist intracellularly in systemic

TABLE 4 Selected List of Biologic Effects Due to Lactic Dehydrogenase-Elevating Virus Infection in Mice[a]

- Increases plasma lactic dehydrogenase and isocitric dehydrogenase by 500-1,000 percent
- Increases other plasma enzymes (malic dehydrogenase, glutamicoxalacetic transaminase, phosphohexose isomerase, glutathione reductase, aspartate transaminase, and others)
- Increases serum gamma globulin level
- Enhances antibody responses
- Delays allograft rejection
- Depresses phagocytosis
- Decreases turnover of plasma proteins
- Increases growth of transplanted tumors
- Decreases mammary tumor incidence caused by the Bittner agent
- Protects against whole body X-irradiation

[a]From Riley (1974).

macrophages. Compromises in integrity of the reticuloendothelial system may precipitate active disease. Conversely, the latent infection can dramatically alter host response to many agents, including LDV, mouse hepatitis virus, and ectromelia in mice and experimental malaria in rats.

Other infections have been reported to alter immunologic responsiveness (Hirsch *et al.*, 1969, 1972; Hotchin, 1971; Hanna *et al.*, 1973; Profitt *et al.*, 1973), change the susceptibility to respiratory carcinogens (Hanna *et al.*, 1973; Nettescheim *et al.*, 1974), and induce increased formation of hepatic microsomal enzymes (Windman *et al.*, 1965; Budillon *et al.*, 1972).

SUMMARY AND CONCLUSIONS

We have attempted to sample the evidence from diverse disciplines showing that environmental factors affect biologic responses of laboratory animals to experimental stimuli. The evidence is overwhelming--greater in some fields than others--but always sufficient to convince even the skeptic that virtually any biologic parameter that can be measured in a laboratory animal may, under the right circumstance, be altered greatly by relatively minor differences in housing practices. By far the areas providing the largest number of examples of known environmental effects on biologic responses were the hepatic microsomal enzyme, endocrine, and immune systems. This is probably a reflection of the sensitivity of the methods employed in these fields, but also an indication that these systems are particulary sensitive to the effects of environmental influences. Nevertheless, all of the examples presented can represent no more than the tip of the total iceberg, which will be revealed by the ever-increasing sophistication and precision of modern science.

We have recommended a concept of the laboratory animal for modern science that integrates the classic view of uniformity based almost exclusively on genetic control, with the emerging view that environmental control can be almost, if not equally, as important. We believe this more balanced view to be far healthier for the modern scientist, because it gives him a much greater appreciation of many factors affecting the validity and reproducibility of his data.

To say that these considerations have important implications for quality control in laboratory animals in the future is at best a serious understatement. The ever-increasing sophistication in modern science simply demands a continuous reevaluation of the precision of all tools essential to the conduct of that science. In practical terms, this means the continued use of time-honored genetic controls coupled with greatly improved environmental controls, including improved design of animal environments, more intensive monitoring of these environments, and more intensive surveillance of physiologic and health status of the animal--from zygote to senescence.

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Social Behavior in a Laboratory Environment

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One objective of ILAR is to encourage production of high-quality animals for research and testing. The definition of "high-quality" would require volumes; thus I will restrict my comments to the behavioral determinants of quality.* However, setting forth this restriction accomplishes less than might appear. Behavior is the activity that results from sensing the environment and then responding in terms of maintenance of homeostasis. The first response is behavioral and the second is physiological.

For perspective on the complexities of assessing behavioral responses in the laboratory, consider a ground squirrel in its natural habitat. Ground squirrels originated in Siberia, but some species have moved to southern Asia and North America, where they may live in hot deserts. They have splendid physiological adaptations of estivation and hibernation, so that they can avoid extremes of heat and cold (and aridity), but the defenses of active ground squirrels against heat and cold are very weak. For example, if left in a trap on a hot day, the squirrel will soon salivate and spread the moisture over its head with its paws. In as short a time as 15 minutes, the squirrel becomes inactive and dies. In nature, this overheating does not occur because the squirrel, when it gets hot, goes into its burrow, which is perfectly controlled for temperature and moisture. But in the laboratory, the animal cannot by some behavior avoid a stressful circumstance. The objective, therefore, of laboratory animal management is to provide an environment that the animal need not attempt to avoid. Inability to escape an environment will precipitate physiological responses to a stress. In nature, animals usually have opportunities to avoid a stress and thus only under certain circumstances show physiological responses to it.

A corollary purpose of ILAR is to develop guidelines for care of animals. Knowledge of behavior is essential for understanding what constitutes good care. As will be seen, we really have little objective evidence for defining "good" care. For example, readers of this review, whether they be veterinarians in charge of colonies or commercial producers of animals, will hope for some practical recommendations for cage size. Unfortunately, research has not produced enough comparisons to allow many specific suggestions. I shall return to this deficiency later. Moreover, the conditions of rearing and maintaining animals are very specific, and an animal's living conditions may influence research or testing involving that animal. Animals reared under different conditions may not respond similarly to identical testing procedures. Therefore, an organism's conditions of rearing must be stated and certified just as much as its genetic source or its diet.

*This review is restricted to laboratory animals, and it will emphasize the behavior of each species under captive conditions. Physiological responses will be noted, but mechanisms will not be discussed, because reviews are available (Conalty, 1967; Perry and Rowlands, 1973; Eleftheriou and Sprott, 1975).

ENVIRONMENT

Physicochemical Factors

Temperature is a major environmental influence, because animals will seek a place where the temperature suits their needs of the moment. In cages, little opportunity exists to change location, and thus the room must be maintained at a suitable temperature. Bedding or a refuge will allow some choice. Lamentably few compara-

tive data are available for determination of the best temperature. For example, tradition says that house mice breed best at about 25°C, but where is the documentation to support this supposition?

Light affects animal behavior in a very complex manner, acting several ways upon endogenous rhythms. The wavelength of light could affect behavior, but no behavioral differences have been noticed in laboratory animals using light suitable for humans. The duration and timing of the light, however, have immense behavioral consequences. A bewildering set of interactions occur among the duration of light, the intensity of light, and the time of day. For example, male house mice are more aggressive just before lights go off than at other stages of the daily lighting sequence (Ziesenis *at al.*, 1975). The actual hour of the day is not important.

Mice kept in constant light have longer estrous cycles than those housed in alternating 14 hours of light and 10 hours of dark. In one study (Campbell *et al.*, 1976), 13 percent of adult females in isolation in constant light have been shown to have cycles of less than 4 days, whereas 41 percent of those in alternating light and dark have been shown to have cycles of less than 4 days. Light intensity itself has not been shown to have direct effects (except at absurd levels), but it may alter the endogenous rhythms described below.

Air is usually taken for granted, since, like humans, most laboratory animals breathe air that has a constant and sufficient supply of oxygen. Thus the animal need not seek a part of the cage that has proper air. However, toxic substances may accidentally enter the room and the animal can do little to avoid them. The regulations for odors in animal rooms are designed to protect the sensibilities of inspectors and visitors rather than the welfare of the animal. A very significant set of chemicals, called pheromones, is carried in the air. These substances, only recently recognized to exist in mammals, are secreted by one individual and affect another of the species. Bronson and Chapman (1968) observed that the estrous cycles of female mice that were kept together, in contrast with those in isolation, were depressed. Their findings are set forth in Table 1. The suppression was obtained by transferring soiled bedding from cages of crowded mice to cages of isolated mice. In contrast, odor from males stimulated estrous cycles. Sattler (1972) exposed isolated mice to the odors of mice in groups and in isolation. The adrenal glands of the mice exposed to air from grouped mice were the largest, as summarized in Table 2. In a different aspect of behavior, removal of olfactory bulbs inhibited aggressive behavior (Ropartz, 1968). The nature of air is thus an important feature of the social environment. We should be cautious in recommending frequent changes of air in circulation.

Noise can be physically damaging, or it can be a signal for some pattern of behavior. Presumably the noise in animal rooms never is sustained at a level that damages the hearing

TABLE 1 Frequency of Estrus in Crowded and Isolated Mice[a]

Living Condition	Frequency of Estrus[b]	Reproductive Status	Adrenal Weight (mg) Isolated	Grouped
Isolated	2.74	Intact	5.57	5.15[c]
Grouped (no males)	1.92	Sham ovariectomized	5.67	5.04[d]
		Ovariectomized	5.02	4.67[e]

[a] Adapted from Bronson and Chapman (1968).

[b] Measured as the number of cycles in 14 days. The frequency of estrus is reduced by placing females in groups of six, even in the strain (C57BL/6J) that responds to grouping, because females have smaller adrenal glands.

[c] Significant at $p < 0.005$.

[d] Significant at $p < 0.01$.

[e] Significant at $p < 0.05$.

mechanisms of mice or humans. However, it has long been known that high levels (95 db) can cause reproductive organs to regress (Zondek and Tamari, 1967). A 1976 report (Chesser *et al.*, 1976) showed that airport noise (105 db) affected the adrenals of wild house mice; these data are provided in Table 3. Fire alarms may inhibit estrous in rats (Gamble, 1976). Another aspect of noise has been so recently discovered for laboratory animals that little can be said. The vocalizations of animals signal certain behaviors, and in young mice ultrasonic sounds alter the maternal behavior. Whether or not laboratory noise masks these effects is unknown.

A final element of the physicochemical series of factors is the diet, in the sense of nutrition and of quantity. Of course, nutrition has been studied for a century for its effects on growth and reproduction. It affects behavior severely by debilitation or by specific conditions of deficiency. Thus, reproduction sometimes is affected by deficiency of vitamin E, and behavioral changes follow. Similarly, the quantity (calories) of food must be sufficient (Chou and Lee, 1964; DeLost, 1975) for reproduction and other behavior patterns. It is assumed that laboratory animals have a sufficient diet so that, like oxygen in air, it is always adequate.

Social Environment

For laboratory animals, the social environment is greatly altered from the natural. It in-

TABLE 2 Effect of Air from Crowded Mice on Adrenal Glands of Isolated Mice[a]

	Living Conditions		
	Crowded (6 per cage)	Experimental Pairs	Reference Pairs
Mean weight of paired adrenal glands (mg)	4.8[b]	5.8[b]	3.9
Mean terminal body weight (g)	28.5	27.9	29.9
Mean number of offspring per female	1.8	6.7	7.6

[a] Adapted from Sattler (1972).

[b] Significant at $p < 0.05$.

TABLE 3 Relation of Adrenal Weight of House Mice to Noise Levels and to Place Captured[a]

	Adrenal Weight (mg)			
	Laboratory		Wild-caught	
Sex	105 db Noise	Laboratory Noise	Airport Noise	Rural Noise
Males	3.3	2.4	2.5	1.9
Females	4.3	2.5	4.2	2.6

[a]Adapted from Chesser *et al.* pz 48:4 (1976), pp. 323-325: 1 table. The University of Chicago Press, publisher.

cludes members of the same species (called "conspecifics"), members of other species, often in the same room, and humans. It lacks predators, unless the person who catches an animal is considered a predator.

The relations with conspecifics are very unnatural. The age and sex composition in a cage differs from natural ratios, and the ability to disperse is usually frustrated. Existing behavioral patterns are exaggerated; new patterns do not emerge. A principal feature of the social environment is density, often used interchangeably with the word crowding; however, these two terms are not analogous. Density refers to the number of animals per unit of space; crowding is a perceived condition of lack of space (Stokols, 1972). Thus, anesthetized animals could be densely grouped but not crowded.

The grouping of conspecifics may result in severe fighting during the establishment of a social organization, whereas placing animals of different species in the same room, even in adjacent cages, probably has no social consequence. Although data are not available, I doubt that placing cats next to mice would have any effect after a few hours of habituation. Humans create noise, move cages and racks, and in many other ways disturb animals. Presumably, the animals promptly learn that the opening of a door means that commotion will follow. Mason (1959) has shown that the corticosterone levels of macaques are significantly lower on weekends, when human disturbance is minimal, than during the week.

TABLE 4 Adrenal and Testicular Response of Mice to Presence of Conspecifics[a]

Mice per Group[b]	Percentage of Testicular Weight to Body Weight	Percentage of Adrenal Weight to Body Weight
1	0.286	0.0156[c]
2	0.303[c]	0.0177
4	0.292	0.0178
8	0.281	0.0173
16	0.279	0.0173
32	0.279	0.0171

[a]Adapted from Bailey (1966). Reproduced by permission of the National Research Council of Canada from the *Canadian Journal of Zoology*, Volume 44, pp. 1007-1012, 1966.

[b]Density constant at 1 mouse/100 cm.

[c]Significant at $p < 0.05$.

The environment includes the physical structures in which the animal lives. Two kinds of enclosures are involved, the room and the cage. I am not aware of any data relating differences in laboratory animal behavior to an aspect of the room such as size, color, or shape. For cages, some information is available.

Bailey (1966) kept male mice in groups of 1, 2, 4, 8, 16, and 32 in cages of increasing size and thus maintained a constant amount of space per mouse. The size of the adrenal glands, relative to body weight, in mice housed in groups of 2 or more was increased over that in mice housed singly, as enumerated in Table 4. There was no statistical difference in adrenal size relative to body weight among the groups of 2 or more, presumably because the density remained the same. A somewhat similar result was obtained using brain protein as a measure of behavior (Bell *et al.*, 1971). Having cages adjacent to each other may result in behavioral interactions. Bronson and Eleftheriou (1965a) showed that placing a wire cage containing a dominant mouse next to one with a naive mouse resulted in adrenal enlargement in the naive mouse. Presumably this change would not occur with solid cages.

Materials of which cages are constructed have not been evaluated for their influence on behavior. The work of Bronson and Eleftheriou (1965a) would suggest that cages with solid walls are preferable, but the evidence is slim. Differences in bedding or flooring of the cage have rarely been examined for their impingement on social interactions (Porter, 1967). Because of the importance of pheromones, it would seem undesirable to change bedding frequently. The difference between gridded (wire) or solid (wood or plastic) floors has not been considered from a behavioral viewpoint, and I doubt that it makes a difference. One experiment was conducted to evaluate automatic flushing devices (Hickey and Tompkins, 1975). The cages studied had wire over paper, wire over flushing, or solid material with bedding. The endpoint measured was the median lethal dose for oral pentobarbital. The results were 128 mg/kg, 127 mg/kg, and 185 mg/kg, respectively. The solid cage produced the most favorable conditions. However, this experiment is a meager contribution to our knowledge.

In studying the social behavior of the laboratory animal, the effect of the caretaker must be considered: For example, rats recognize their caretaker by olfactory cues (McCall *et al.*, 1969).

Early Experience

An aspect of behavior that has only recently been observed is the relationship of the behavioral experiences in early life to the condition of the housing and grouping (Cosnier, 1967). Sometimes the expression of this relationship does not

appear until adulthood--progeny of stressed parents are less active, at least in early life.

Hockman (1961) applied electric shock to 16 pregnant rats, but not to 16 others. The progeny were cross-fostered, so that each female suckled young from a shocked (stressed) and a nonshocked parent. The young born to shocked females were more active in an open field at 30-45 days than were the other young. However, no difference existed at 180-210 days. The same results were measured when female mice were crowded, a more natural stress than electric shock (Keeley, 1962).

Very brief handling may affect behavior. Manipulating rat pups for 3 minutes a day for 20 days alters their adrenal functions (Denenberg and Smith, 1963). Early experience may also alter copulatory behavior in the adult mouse (Ward, 1972). Finally, grouping and handling interact to produce gastric lesions (Ader, 1970) and abnormal progeny. Hamburgh *et al.* (1974) have shown that resorptions and abnormal embryos are greater in the grouped mice (18 pregnant mice housed in a cage of 17.5 × 17.5 × 25 cm) than in controls (2-4 pregnant mice housed in the same size cage) as shown in Table 5. Abnormal behavior results from various treatments in infancy (Fox, 1968).

TABLE 5 Changes in Resorption and Reproductive Abnormalities in Mice Housed in Groups of 18[a]

	Number per Cage	
	2-4	18
Percentage of resorption	0	4.2
Percentage of abnormal embryos	0.75	9.5
Adrenal weight (mg/10 g body wt)	3.04	4.6
Total mice	13	36

[a]Adapted from Hamburgh *et al.* (1974).

Circadian Rhythms

The environment, through the mechanism of length of daylight (photoperiod), affects rhythmic activities in very complex ways. The endogenous rhythms of plants and animals initiate and act upon numerous behavioral and hence physiological responses. To attempt an adequate description of such rhythms is impossible in this review; for reference, proceedings of several symposia are available (Brown *et al.*, 1970; Menaker, 1971). The essence of the phenomenon may be defined as follows: Organisms have endogenous rhythms of activity that continue in constant conditions of photoperiod and temperature and have a duration approximately 24 hours (circadian), 24.8 hours (circumtidal), 28 days (circumlunar), and 12 months (circannual). Fortunately, the laboratory animal profession usually need be concerned with the circadian cycle alone. The behavioral aspect to notice is that a mouse, for example, can and will shift its activity gradually in response to a sudden change in length of day, hours of day, or intensity of light. Unfortunately, laboratory animals (mice, rats) have rarely been used for research on circadian rhythms; thus, much of our knowledge comes from chaffinches, flying squirrels, house finches, pocket mice, starlings, insects, and bean plants.

Let me create a sequence for a laboratory animal to illustrate the behavioral responses. Suppose that we place a mouse in a cage in a room and alter the lighting conditions in various ways. First, consider the case of lights on from 0800-2000 hours during which time the mouse will be active night after night. Now change the timing of light abruptly to 1600-0400. The mouse will *gradually* (over 5-10 days) shift its activity to the hours of darkness. Next, maintain light all 24 hours at an intense illumination (120 lux). The mouse will *delay* its activity period each day by 5-15 minutes. But if the light is dim (5 lux), then its activity will *advance* each day. Finally, in constant darkness, the mouse will delay its activity a few minutes each day.

For laboratory animals, the significance of circadian cycles is that rhythmic changes comprise part of physiological processes as well as behavior. The most frequently measured response is the circadian rhythm of adrenal activity, as indicated by levels of corticosterone (Haus and Halberg, 1970). The corticosterone reaches a peak just before initiation of activity and about 4 hours after the peak of adrenocorticotropic hormone (ACTH). Reversal of the photoperiod reverses the peak in about 5 days. A more complex relation involves the results of grouping (Ader and Friedman, 1968). Rats, behaviorally stressed while social organization is being formed, when killed at the crest of the corticosterone cycle, had a level of corticosterone 1.5 times that of nonstressed rats. However, rats killed at the trough of the cycle, had a level of corticosterone 5 times that of nonstressed rats. Thus, physiological responses to behavior differ during the 24-hour period according to the amount of available light.

The existence of circadian rhythms requires that researchers take a few precautions. The first is that the lighting conditions must be known and specified. If a change occurs in an animal's photoperiod, then no experiments should be conducted with the animal for at least a week. Also, measurements must be obtained at the same time to take into account the circadian clock; if possible, animals should be measured several times.

A practical problem is the consequence of a power failure. For reasons too complicated to describe here (see Follet and Sharp, 1969), interruption of a period of light by a period of darkness has no effect. But one should be sure to reset the time clocks promptly. In contrast, a burst of light lasting even a few seconds in some cases will skew endogenous rhythms and set physiological processes in motion. Light switches and doors should be locked to prevent accidents from happening.

SOCIAL BEHAVIOR

So far we have considered some environmental influences that confront animals in laboratories. Now we turn to a more direct discussion of behavior, emphasizing the nature of behavior in laboratory animals and its consequences.

As one would expect, the genotype affects behavioral results. For example, several strains of mice have been tested for frequency of aggression when grouped 2, 4, or 8 mice per cage. In some cases, the attacks per mouse increase, but in others they do not (Vale *et al.*, 1971). The adrenal response also varies. Mice can be programmed in their development to be aggressive when suitable conditions occur. Mice raised in isolation fight at their first exposure as an adult to another mouse (Valzelli, 1969).

Social Rank

In nature, animals organize their populations into social systems that conserve resources and tend to assure that at least a few individuals survive a period of scarcity. The systems at their extremes are called *territory* (the defense of an area) and *social rank* (the arrangement in an order). During the past 50 years, a wealth of information has accrued showing an infinity of variations on the general theme (Brown, 1975). It should be noted that in many species (house mice, macaques, and others) the two systems form a continuum, with intermediate stages that can be called territorial rank. Furthermore, in laboratories, an animal has little chance to defend a territory for long. Studies of mice in a room (Davis, 1958) have shown that at low densities male mice defend territories successfully, but at high densities the organization shifts to a rank. The reverse can occur. Groups of five male mice transferred from cages having 1.3 m^2 floor area to cages having 2.2, 3.8, or 5.2 m^2 have shown changes in dominance in 9 of 13 groups (Poole and Morgan, 1976). The dominant mouse uses most of the floor area as a territory, and, in the largest cage, even some subordinates hold a territory. In laboratories, the animals can only manifest territorial behavior by fighting animals in adjacent cages. However, provision of cover or baffles can permit the formation of territories (Mackintosh, 1970, 1973). Apparently an aversive pheromone is produced in isolation and lost in grouping (Jones and Nowell, 1974).

Another aspect of social rank is noteworthy for animal care. In nature, low-ranking or recently defeated animals can run away and escape from dominant individuals. But in cages, the loser has to stay and be beaten at the whim of the victor. Thus, the loser is persistently subjected to stress. The physiological consequences of this tension are discussed below.

The arrangement of a social rank in a newly formed group of animals follows a definite sequence in nature and in captivity. First, the animal notifies others that it intends to assert its position. In mice, the process is to adopt a posture of hunched back, take short steps, and vibrate the tail. Next, if the mouse stands its ground, a stage of sniffing and vocalizations constitute threats. Finally, if its antagonist still has not retreated, the mouse attacks its opponent. The fight may be severe, sometimes resulting in death. In other cases, the loser can escape or avoid the victor and survive. The behavioral patterns in this sequence are labeled as aggressive, but they cannot be considered belligerent in the same sense as human aggression, because we know nothing of the mental or motivational state of the animal. Another distinction to be made is that an animal that bites (or tries to bite) a caretaker is defensive, not aggressive. The word "aggressive" should be restricted to behavior associated with social organization. The behavioral result of dominance-seeking is the arrangement of animals in a rank. The physiological repercussions are extensive and pervasive--the repercussions can be united under the word "stress." Physiologically, the effects are the same as those from such other stresses as restraint, noxious agents, or cold. The degree, of course, differs according to circumstances.

Before discussing some physiological consequences of stress in laboratory animals, let us look at data that concern the notion that behavior is a stress. The original demonstration (Christian, 1955) that grouping mice results in an increase in adrenal weight has been followed by innumerable experiments, especially after simple ways to measure corticosterone were introduced. The extensive work is reviewed in detail (Bronson, 1967; Archer, 1970b; Christian, 1971, 1975), and many other examples are available.

In 1957, Davis and Christian showed that the top-ranking male mouse in a group of six had adrenal glands indistinguishable in size from an isolated male, whereas lower-ranking mice had increasingly large adrenals, even when corrected for loss of body weight. Ten years later, Louch and Higginbotham (1967) confirmed these findings by measurement of plasma corticosterone; their results are reported as Table 6. The effect of defeat has been measured physiologically also. In one experiment (Bronson and Eleftheriou, 1965b), the adrenals of a mouse defeated by a trained fighter weighed 7.3 mg; adrenals of a mouse never defeated but exposed to a fighter through a partition weighed 3.2 mg; and a mouse defeated previously and then exposed through a partition had adrenals weighing 11.1 mg. A situation more closely approximating laboratory conditions contrasted the adrenal response of mice individually caged but separated by either wire or solid wood partitions; those findings are reported in Table 7. Archer (1970a) showed that success in an encounter failed to increase the size of the adrenal glands (see Table 8). No differences were found in adrenal weight, although ascorbic acid may have been depleted (differences not significant at 0.05 level).

The hierarchical arrangement perhaps can be produced in response to odors from crowded mice (see Table 2). Note that the range for adrenal weights of crowded mice is wide and includes some within the range of the reference animals. Presumably the individuals with small adrenals

TABLE 6 Mean Adrenal Weights and Plasma Corticosterone Concentration in Dominant Mice[a]

Treatment[b]	Number	Corticosterone (µg/100 ml plasma)	Adrenal Weight (mg/g body wt)
Isolated (control)	35	9.3	0.16
Dominant	8	11.9	0.19
Subordinate	24	19.9	0.21

[a]Adapted from Louch and Higginbotham (1967).

[b]Mice were isolated after weaning and then placed in groups of 4 for 24 hours.

are in the top social rank of the crowded group. But the range for the mice receiving air from crowded cages (the experimental pairs) does not include any individuals with small adrenals. Presumably, all are subordinate, even though isolated, due to the effect of air from crowded cages. It is possible that in a room the presence of crowded mice may affect the uncrowded. A mouse can distinguish between the odors of victors and victims, as summarized in Table 9, or of novel mice (strangers). Also, in mice of subordinate rank, results show greater fluctuations in corticosterone levels than in dominant mice exposed to an open field (Chapman *et al.*, 1969), but only if they have been handled as infants.

Disruption of social rank, resulting from frequent shifts of individuals, has been shown to have severe effects on adrenal activity. McKinney and Pasley (1973) kept mice in singles or in groups of three, and some of each were moved to different cages. For mice alone in a cage, shifting to another cage had no effect on adrenal weight. In mice in groups, the dominant mice had smaller adrenals than the subordinates; subordinates shifted daily to another group had the largest adrenals. Mice introduced into a crowded cage showed increases in corticosterone levels (Del-Pup and Palmes, 1971).

A recurrent problem that is important to ecologists, but which may have little interest for the person in charge of laboratory animals, is to what extent such behavioral effects occur

TABLE 7 Mean Adrenal Weights and Concentrations of Adrenal Ascorbic Acid in Caged Mice Separated by Wire or Solid Wood Partitions[a]

Partition[b]	Number	Adrenal Weight (mg/100 g body wt)	Adrenal Ascorbic Acid (µg/100 g body wt)
Wire	22	15.2	103.4
Wood	23	13.4	120.3
Probability	--	0.1-0.05	<0.05

[a]Adapted from Archer (1969).

[b]Male mice were arranged in wire cages in tiers of eight or separated by wood partitions for 14 days.

TABLE 8 Relationship Between Success in Aggressive Encounters and Stimulation of Adrenal Glands[a]

Preliminary Exposure	Treatment	Adrenal Weight (mg/100 g body wt)	Adrenal Ascorbic Acid (µg/100 g adrenal wt)[b]
Isolated	Isolated	13.8	121.1
Isolated	Defeated daily	16.0	117.4
Exposed to trained fighter	Isolated	13.5	110.1
Exposed to trained fighter	Won twice daily	13.7	108.4

[a]Adapted from Archer (1970a).

[b]None of these depletions was statistically significant.

TABLE 9 Time Spent Investigating Odor of Victor, Victim, or Novel Mice[a]

Experimental Condition	Preference for Odor of:[b] Victor	:	Victim
Defeated by trained victor	1	:	15
Same but exposed to novel mouse	15	:	1
No contact possible	12	:	4
No victor (cage only)	12	:	4
Victors	6	:	2

[a]Adapted from Carr *et al.* (1970). Copyright (1970) by the American Psychological Association. Reprinted with permission.

[b]After exposing mice to various experimental conditions, each mouse was exposed to the odor of a victor or of a victim that was either known or novel to the experimental mouse. The number of seconds spent with each odor was determined and then translated into a ratio for ease of comparison.

in nature. One example may be cited from observations of California ground squirrels (Adams and Finn, 1972). A dominant squirrel spends much time sitting erect to survey the scene for other squirrels, and newcomers are immediately subdued. The dominant animal has smaller adrenals (0.550 mg/100 cm body length) than the subordinant (0.760 mg/100 cm). Also, a negative correlation (r = -0.835) exists between the "time in sight" and the weight of the adrenals.

Physiological Results

The social environment affects several physiological functions. The data presented above have concerned the question of behavior as a stress and demonstrate that stress does result from social organization and also some physical stimuli. The physiological changes that follow ACTH elevations are known for many species and can be predicted. In this section, additional examples of physiological and pathological consequences of stress are provided.

Reproductive behavior is strikingly altered by the effects of social organization. Only a few examples will be cited here, because the endocrine consequences of stress are abundantly documented [see Christian (1975) for a review]. As is well known, the reproductive organs are inhibited in low-ranking individuals. Christian and Le Munyan (1958) have shown that lactation is affected by previous crowding (Table 10). Note that the young nursed by previously crowded females gain weight more slowly than do their counterparts nursed by isolated females. This study has also shown that the effects of crowding on lactation can persist for at least one generation after crowding; progeny of a crowded female weigh significantly less at weaning than the progeny of an isolated female. Bronson (1973) has investigated hormonal involvement. Male mice placed four to a cage for 1 hour a day for 14 days show a 500-600 percent increase in plasma cortisol. Concentrations return to base level in 1-3 days in the mice that establish dominance, and in 3-6 days in the mice relegated to subordinance. The level of follicle-stimulating hormone (FSH) decreases 19 percent and the level

TABLE 10 Effects of Crowding and Isolation on Weight Gains in Infant Mice[a]

Treatment[b]	Weight of Pups (g) at Weaning	Probability
Born in:		
crowded environments	8.80	0.10
isolated environments	8.31	
Nursed by:		
crowded females	7.84	0.001
isolated females	9.09	
Nursed by:		
own mother	8.55	0.9
foster mother	8.59	

[a]Adapted from Christian and Le Munyan (1958).

[b]In all cases, 56 litters were examined.

of luteinizing hormone (LH) decreases 94 percent, both hormones being below average at the end of 14 days. Thus, the reciprocal relation exists only for the first few days after grouping. Conversely, conditions and number of rats in a cage may affect the assay of gonadotropins using ovarian weight as a measure (Chance, 1956). Lamond (1959) has demonstrated that grouping mice can result in anestrus. Isolated females show twice as many estrus cycles as grouped females.

The release of LH-releasing factor, measured by loss of ascorbic acid, is influenced by defeat, a phenomenon summarized by Table 11 (Eleftheriou and Church, 1968). Copulatory behavior is affected by rank, as shown in Table 12, which reports the results of training male mice to be aggressive or submissive and then exposing them to a virgin female (Kahn, 1961). During lactation, aggressive behavior is suppressed (Thomas *et al.*, 1970).

One important aspect of laboratory animal management is the optimal production of progeny (Perry and Rowlands, 1973). Christian and Le Munyan (1958) have shown that if 10 pairs of mice are crowded into one cage, the dams bear less than one-third the number of pups borne by dams housed

TABLE 11 Effect of Victory or Defeat on Depletion of Ascorbic Acid[a]

Treatment	Percentage of Depletion[b]
Controls	18.6
Defeated days	
1	19.2
2	11.2
4	3.4
8	1.8
16	2.2
Victorious days	
5	9.1
6	17.8

[a]Adapted from Eleftheriou and Church (1968).

[b]A low percentage of depletion indicates low activity of LH-releasing factor.

TABLE 12 Patterns of Copulatory Behavior of Male Mice Trained to Be Aggressive or Submissive and Then Exposed to Virgin Female Mice[a]

Behavior Pattern	Behavior Performed by Number of:[b] Aggressive Males	Submissive Males
Pursue female	1	0
Copulate	7	0
Mount female	9	1
Groom female	11	6
Nudge female	1	17
Inactive	0	11
Run from female	1	35

[a]Adapted from Kahn (1961).

[b]A total of 30 aggressive and 70 submissive mice were tested.

one pair per cage. In a more comprehensive study, the differences also are great as seen in Table 13. As a digression, one might inquire about the optimal number of females per cage when judged by costs. Clearly, the most prolific reproduction is achieved with one pair per cage, but washing, cleaning, and initial costs are higher for one pair than for several pairs. A model for computer simulation could be developed to maximize the rate of reproduction and minimize the cost per weaned mouse. I suspect that three females per cage might be the ideal number.

Most of the research cited above deals with the effect of caging in groups or with disturbance on the activity of the adrenal cortex and subsequent effects on reproduction. Yet, the effect of ACTH on behavior also needs to be considered. Brain *et al.*, (1971) indicate that ACTH increases aggression and that dexamethasone decreases it.

TABLE 13 Comparisons in Reproductive Performance in Crowded and Isolated Mice[a]

Measure	Crowded Pairs	Isolated Pairs	Significance
Implantations	7.70	9.97	*c*
Litter size	6.27	6.85	*c*
Embryos resorbed	0.175	0.141	*b*
Ova lost	3.71	3.96	
Percentage of male offspring	48.30	52.80	*c*
Weight of adrenal gland (mg)	5.70	3.90	*d*
Density of sperm (thousands/mm)	447.0	589.0	*c*

[a]Adapted from Snyder (1966).

[b]Significant at $p < 0.05$.

[c]Significant at $p < 0.01$.

[d]Significant at $p < 0.001$.

But recent reports confuse the situation by indicating that ACTH decreases aggressive behavior (Poole and Brain, 1974). A complicated set of experiments indicates that the ACTH rather than gluccorticoid or testosterone is responsible (Leshner *et al.*, 1973). In addition, the role of the adrenal medulla cannot be neglected, but limitations of scope prevent a comprehensive treatment here (see Welch and Welch, 1969; Eleftheriou and Sprott, 1975).

Response to drugs will vary with behavioral episodes in several fundamental ways (Ellis, 1967; Palmes and Del-Pup, 1970; Baer, 1971). The existence of endogenous rhythms of activity is responsible for certain changes in adrenal function during 24 hours. In addition, there are monthly fluctuations in corticosterone levels and in the amplitude of the cycle, as explained in Table 14. The response to housing varies at the peak and trough of the cycle (Ader and Friedman, 1968). However, prior handling (early experience) does not produce changes in adrenocortical function when adulthood is reached (Grota and Ader, 1970), perhaps because the adrenocortical rhythm has not yet been established at the time of handling. Drugs are influenced by behavior because, for example, aggressive behavior leads to stress, which in turn affects physiological responses. Few documented examples of behavior impinging upon action of drugs in laboratory animals exist, because most of the research considers the reverse: the action of drugs on aggressive behavior. Nevertheless, we can be confident that behavior affects response to drugs.

Resistance to infection changes with changing levels of adrenal steroids, and social environment, acting through the pituitary adrenal axis, has been shown to alter resistance (Ader, 1967). For example, susceptibility to parasitic infections has been manipulated. Male mice were isolated until they became sexually mature and then were placed in groups of six with isolated males serving as controls. Each mouse of a group and each of the isolated mice were put in a clean jar for

TABLE 14 Monthly Fluctuations in Corticosterone Level and Monthly Cycles in Male Mice[a]

Month	Mean Level of Corticosterone (μg/100 ml serum)[b]	Ratio of Peak to Trough[b]
January	15.2	7.8:1
February	21.2	8.8:1
March/April	15.6	6.0:1
May	11.2	7.3:1
July	14.3	5.8:1
August	6.0	3.2:1

[a]Adapted from Haus and Halberg (1970).

[b]Isolated male mice were measured several times a day. They were housed at 20°C and in 12 hours of light followed by 12 hours of darkness.

TABLE 15 Severity of *Trichinella* Infections in Isolated and Grouped Male Mice[a]

	Isolated	Grouped[b]
Number of mice	11	11
Adrenal weight, mg	3.92	4.19
Percentage with adult *Trichinella*	27	100
Number of mice	6	5
Adrenal weight, mg	4.24	5.08
Maximal larvae	1,273	1,733
Minimal larvae	880	1,433

[a]Data from Davis and Read (1958).

[b]For 4 hours a day; measurements were taken 10 days after oral dose of *Trichinella*.

4 hours each day for 10 days and then returned to its own jar. All mice received the same dosage of *Trichinella spiralis* intragastrically. The results, given in Table 15, showed higher levels of infection in grouped mice. The responsible mechanism was presumably the reduction of inflammatory response of the wall of the gut. Social environment also has been linked to concentration of antibodies in the blood. Mice were arranged in groups and injected with beef protein. Antibodies rose to a peak on the eleventh day after injection and declined to the base line on day 28. The maxima (log of titer) in four groups of mice were 0.98, 0.66, 1.10, and 1.15. The maxima in two batches of isolated mice were 1.55 and 1.45 (see Table 16). The dominant mice in each group showed a log of titer at 1.35, whereas all the others reached no more than 0.84. Thus, dominant individuals showed a "normal" antibody reaction to beef protein.

It is well known that numerous pathological conditions are triggered by stress (Albert, 1967). Here it is desirable to point out that the social environment, particularly an overpopulated one, can produce gastric lesions and glomerularsclerosis (Christian, 1963). Ulcers or lesions frequently develop in mice or rats subjected to psychological tests such as shock avoidance (Ader, 1970). Renal lesions occur only in subordinate mice. Mice in dense populations have higher arterial blood pressure (121 mm Hg) than do isolated controls (107 mm Hg) (Blaine, 1973). The development of gastric and renal lesions in laboratory animals has been essentially ignored, so that few data are available. Ascitic tumors decrease in mice kept in groups, in contrast to those in isolation, but the strain of mouse used in the experiment responds to crowding with *reduced* adrenal size. Injecting ACTH increases the size of the ascitic tumor (Dechambre, 1971).

Ectoparasite loads may result from behavioral disturbances related to adrenal function. Continual disturbance of a rank is known to activate the pituitary axis (McKinney and Pasley, 1973). Disturbing the social hierarchy can predispose mice to severe infestations of lice (Lodmell *et al.*, 1970). A decrease in mutual grooming surely is partially responsible, but the presence of an eosinopenia indicates that adrenal deficiency may also account for the increased susceptibility.

One topic that has attracted much attention in the literature is called "emotionality." It is measured by an animal's level of activity and frequency of urination or defecation when it is placed in an "open field" (a large cage, usually with grids painted on the floor). Presumably, any truly novel situation would evoke the same reactions. Chapman *et al.* (1969) have used plasma corticosterone concentrations as a measure of emotionality in mice. They have shown an equal rise in plasma corticosterone concentrations among mice (whether dominant or subordinate) placed in an open field, if the mice have not been handled in infancy. If the mice have been handled in infancy, however, subordinate mice will show a significantly greater rise in plasma corticosterone concentrations than dominant mice. For a person managing an animal colony, the significance of this experiment is that seemingly minor aspects of animal housing and care may affect the outcome of a future experiment.

Several studies have investigated the emotionality of aggressive and nonaggressive animals (i.e., winners and losers). First, good correlations exist between measures of aggression (winning) and such behaviors as sniffing, biting, or vibrating the tail. Also, a positive correlation exists between emotionality and plasma sodium concentration (Brain and Nowell, 1970). Yet no difference exists in the adrenal response of winners and losers to the stress of ether administered after a fight (Brain and Nowell, 1969). In rats, density significantly alters many measures of emotionality (Morrison and Thatcher, 1969). Exposure to a stressful stimulus will alter plasma corticosterone, growth hormone, and prolaction concentrations (Brown *et al.*, 1974).

EXPERIMENTAL RESULTS

The data below are organized according to species for the person primarily interested in one or two laboratory animals. Within each species, the data to the extent possible will be discussed--first about the environment and second about behavior.

House Mice

Because the mouse is a highly popular animal for research, much of the data have been discussed above. However, a few additional items merit mention. Aggressive behavior develops about 35 days after birth (McKinney and Desjardins, 1973). Copulatory behavior develops later (40-95 days). Little has been said about the effect of housing or density on females; because males demonstrate aggressive behavior more vividly, females have been neglected. But recent work, mostly unpublished, shows that females are equally aggres-

TABLE 16 Antibody Response of Group and Isolated Mice Injected with Beef Protein[a]

Grouped	Injected	Mean Body Weight (g) Before	Mean Body Weight (g) After	Mean Adrenal Weight (g)	Log Titer at 11 Days
Yes	Yes	22.8	22.2	3.89	0.98
Yes	Yes	27.4	30.6	4.29	0.66
Yes	Yes	23.2	22.0	4.34	1.10
Yes	Yes	31.8	31.8	5.01	1.15
Yes	No	22.7	25.3	4.20	--
No	Yes	24.3	30.2	2.95	1.55
No	Yes	27.4	31.8	3.35	1.45
No	No	24.8	33.4	3.12	--

[a]Adapted from Vessey (1964).

sive, although their aggressive behavior differs from that of males. An isolated female has heavier adrenals and ovaries, lower plasma corticosterone, and more frequent estrous than a grouped female. The single male has lighter adrenal glands, as summarized in Table 17.

Mice have been the species of choice of research on pheromones, mostly notably the urinary pheromones of male house mice. These substances stimulate the reproductive system of female mice. Adult females housed individually have estrous cycles of 4-5 days. When several females are housed together, this pattern ceases, as set forth in Table 18. Depending upon the number of females involved, these noncycling animals may enter a state of pseudopregnancy or anestrus. Although this suppression of estrus is poorly understood, apparently pheromones produced by the females are responsible. When male mice are introduced into a group of acyclic females, the females begin cycling again, as shown in Table 19. Interestingly, not only do they cycle, but initially they cycle together, as noted in Table 20. This synchrony is borne out by the high rate of inseminations on the third day after the introduction of a male. In females with normal cycles, estrous receptivity would be expected every 4 or 5 days, and thus, on the average, no more than 25 percent of a colony would be in estrus on a given day. In females grouped and then exposed to males, however, approximately 50 percent become pregnant on the third day. This phenomenon has had its practical benefits for laboratory experimentation in providing a method for maximizing the number of young mice born on a given day.

It is not necessary that the male physically be present to produce the effect in females. Dirty bedding from a cage of males is sufficient, as is air that has been blown over a cage of males, as summarized in Table 21. Thus it appears that the arousing substance is an airborne pheromone. That urine is the source of the pheromone is demonstrated by the effect of male urine placed directly onto the nose of a female. Moreover, castration of a male eliminates the stimulatory effect of its urine, and androgen treatment restores it, indicating the role of

TABLE 17 Adrenal and Preputial Weights of Isolated and Paired Mice[a]

Treatment[b]	Reversal	Mean Left Adrenal Weight (mg/100 g body wt)	Mean Preputial Weight (mg/100 g body wt)
Isolated	Isolated	6.1	130.3
Isolated	Paired	7.7	127.5
Paired	Paired	6.6	113.7
Paired	Isolated	5.8	129.3

[a]Adapted from Brain (1971).

[b]Shifting mice from isolated to paired living resulted in adrenal enlargement. Males were weaned at 18-22 days and kept in isolation to day 30. Then some were kept in isolation and some paired. After 21 days, half of each group was reversed for 21 days. The differences between I-I and I-P and between I-P and P-I were statistically significant. None of the differences in preputial weights were significant, indicating little, if any, effect on androgens.

TABLE 18 Frequency of Estrous Cycles in the Presence of Other Females[a]

Treatment	Frequency of Estrous Cycles[b]
Isolated to group	2.90
Group to isolation	2.57
Isolated	2.33
Group	0.33
Perforated partitions	1.10
Solid partitions	0.97
Blinded, isolated	2.00
Blinded, group	0.56

[a]Adapted from Whitten (1959).

[b]Number of estrous cycles within a 16-day period.

testicular androgen in the production of the pheromone (Bronson and Whitten, 1968).

A second pheromone associated with the urine of the male house mouse is responsible for the "pregnancy block effect." If a recently impregnated female is exposed to a male other than the one that inseminated her, the probability is high that the pregnancy will fail and that the female will resume cycling. Because prolactin administration (which supports corpora luteal function) protects against the block, apparently the strange male induces an alteration in the endocrinological events of pregnancy. Whatever the cause, the fertilized eggs do not implant in the uterus, and thus pregnancy fails (Bruce, 1959). The blocking power of a second male is inversely related to his degree of genetic similarity to the original male. That is, a male of the same inbred strain will fail to inhibit pregnancy, whereas males of certain other strains or wild mice will thwart it. Again, the urine is the source of the pheromone, and the testes are necessary for its production (Bruce, 1965).

A third male mouse pheromone is the puberty-accelerating substance (Vandenbergh, 1973). There is considerable variation in the onset of puberty among female mice. Some of that inconsistency undoubtedly stems from the difficulty of selecting a suitable index for sexual maturation, from genetic differences, and from miscellaneous sources of variation. In addition, the timing of pubertal development is highly susceptible to conspecific social influences.

TABLE 19 Rate of Estrus in Isolated and Grouped Mice after Presentation to Males[a]

Day after Exposure to Male	Grouped Mice Copulatory Plug on Day:	Isolated Mice
1	6	25
2	18	40
3	107	53
4	49	35
5	9	11
Residue	14	41

[a]Adapted from Whitten (1959).

TABLE 20 Vaginal Plugs in Mice Kept in Groups of 28-40 According to Days Elapsed after Copulation[a]

Day	Number of Females with Plugs
1	43
2	44
3	146
4	42
5	14
6+	28

[a]Adapted from Whitten (1956).

Compared to those reared alone since weaning, females reared with other females reach sexual maturity at a later age, whereas those cohabiting with males mature earlier (Bruce, 1962). Pheromones are at least partially responsible for these phenomena. Even dirty bedding from a male will accelerate the development of a female in whose cage it is placed, as demonstrated in Table 22. Puberty has been watched in the female mouse by determining age at opening of the vagina and by examining vaginal secretions for the appearance of large cornified cells indicative of the first estrus (see Table 23).

As briefly noted before, the effectiveness of dirty bedding from males' cages in accelerating puberty in females indicates that the male himself need not be present and that some substance that he has deposited on the bedding is responsible. Tests of urine placed directly onto the nose of a young female indicate that the pheromone leaves the male by way of his urine. The ability of a male to produce the puberty-accelerating pheromone in his urine depends upon the presence of functioning testes, as can be seen from Table 24. Castration eliminates his pheromonal potency, and this potency is restored by exogenous androgen. Further, the male's production of pheromone is in part a function of his housing conditions and dominance status within a group. Dominant males produce more puberty-accelerating pheromone than do subordinate males, presumably as a result of

TABLE 21 Percentage of Females That Came into Estrus from Exposure to Air Blown Over Males[a]

Position Relative to Male	Number of Females	Percentage in Estrus
Upwind	49	35
Downwind	47	68
Under	44	84
Outside	58	25

[a]Data from Whitten *et al.* (1968).

TABLE 22 Initiation of Estrus Following Stimulus of Male or Bedding from Male's Cage[a]

Treatment	Grouped	Single (Age in Days)	Grouped
Male present	39.6	28.0	35.7
Male bedding	42.3	33.0	--
No male	54.6	35.9	55.1

[a]Adapted from Vandenbergh *et al.* (1972).

differences in androgen secretion. An additional consequence of crowding is delayed implantation, but perhaps this effect can be produced solely under experimental conditions. When crowded females (22 per cage) are treated with gonadotropins to stimulate superovulation, a striking number of ova do not implant at the normal time after ovulation (Dickson, 1964).

Significant differences in adrenal weights have been measured among diverse strains of mice in the response to grouping (Theissen, 1963). Six strains tested at three densities show differences in adrenal weights, as given in Table 25, and in variability among strains. Some strains manifest a difference in weight, but others do not. One must not, however, conclude that no change in function occurs, since it is well known that corticosterone concentrations may change without a corresponding change in adrenal weight. No effects of grouping have been observed for the DUB/ICR strain (Anton, 1969). To determine if a winner generally has larger adrenals at the start, mice have been paired for 5-10 minutes, a winner chosen, and the adrenal glands measured. No difference has been found in 78 pairs (D. E. Davis, 1956, unpublished), an observation confirmed by a detailed measurement of size of testes, seminal vesicles, preputial glands, and thymus. Two males have been placed together for 10 minutes and classed as winners or losers, or in some cases, no decision (Lloyd, 1971). No marked differences distinguish the weights of the organs, but in mice in groups of six, the top mouse has the largest organs.

TABLE 23 Onset of Estrus in Mice as Affected by Timing of Presence of Males[a]

Treatment[b]	Vaginal Opening (Age in Days)	First Estrus (Age in Days)
Male present until day 21	30.5	37.1
Male present until day 30	30.4	41.9
Male present until day 38	32.4	45.6
Male absent	34.9	57.1
Male present after day 21	31.8	42.2
Female present after day 21	32.2	48.8
Adult absent	35.2	56.9
Male 20 days before weaning	29.8[c]	39.6
Male 20 days after weaning	32.4[c]	43.6
Male absent	30.4[c]	54.9
Male present	30.9	39.7
Castrate present	39.3	54.6
Cage activated	38.5	58.1
Male behind mesh	31.2	40.6
Male odor in air	29.5	42.3
Solitary male odor	32.7	45.4
No odor	34.9	54.6
1 female per cage	26.1	30.4
2 females per cage	27.6	33.6
3 females per cage	28.1	34.1
5 females per cage	28.7	34.1
7 females per cage	29.7	37.1
9 females per cage	29.9	37.6

[a]Adapted from Vandenbergh (1967, 1969) and Drickamer (1974).
[b]Each group represents one experiment; data should be compared.
[c]Comparisons that lack statistical significance.

Rats

In the genus *Rattus*, more than 400 species or subspecies have been described. Fortunately, we tend to deal solely with the Norway rat (*Rattus norvegicus*). The domesticated Norway rat is not aggressive, perhaps the result of unplanned selec-

TABLE 24 Age in Days of Female Mice at Opening of Vaginal Orifice and at First Estrus; Weights of Mice Exposed to Urine of Various Donors[a]

Treatment	Age of Exposed Female at Vaginal Opening	Age of Exposed Female at Estrus	Weight of Exposed Female in Grams at Vaginal Opening	Weight of Exposed Female in Grams at Estrus	Weight of Exposed Female in Grams at Age in Days 21	28	35
Sham-operated	31.7	33.3	16.7	17.2	9.2	14.4	17.9
Castrated male	32.4	36.3	16.4	17.3	9.3	13.9	17.4
Preputial-ectomized	31.3	33.1	16.8	17.5	9.2	14.8	18.2
Female	35.1	39.8	17.2	18.3	9.4	14.2	17.3
Male rat	31.5	34.2	16.1	16.9	9.4	13.9	17.2

[a]Adapted from Colby and Vandenbergh (1974).

TABLE 25 Adrenal Weights Among Grouped and Isolated Mice of Several Strains[a]

Cage Size, cm	12.5 × 8.75 × 10	25 × 8.75 × 53.75	25 × 8.75 × 106.25
Mice per Cage[b]	1	10	20
Strain	Adrenal Weight in Pairs (mg/100 g body wt)		
C57B7	10.8	11.3	12.2
BALB/c	11.8	12.7	14.2
DBA/2	11.5	10.6	10.4
A	9.9	9.4	9.4
C3H/2	14.8	15.2	10.8
R111	11.5	11.4	12.3

[a]Adapted from Thiessen (1964). Copyright (1964) by the American Psychological Association. Reprinted by permission.

[b]Mice were kept in isolation and at ages 48-51 days placed in groups of 1, 10, and 20 for 28 days in cages of increasing size to give the same area per mouse.

tion by animal caretakers. However, they show some responses to grouping. In rats kept one or four per cage, 3 minutes of stressful stimulation raises plasma corticosterone, and this elevation is maintained for hours in isolated rats but drops in one-half hour in grouped rats (Plaut and Grota, 1971). Handling reduces the plasma corticosterone response of rats to electric shock in infancy and increases the level of histamine in the blood (Cassell *et al.*, 1967). Rats that are not handled in infancy show little elevation of corticosterone in response to trauma until they are 18 days old, whereas rats handled daily from birth respond as early as 3 days of age (Levine, 1968). The age at which the adrenal glands begin to respond to environmental changes can be determined. At day 21, rats show statistically significant differences in corticosterone levels after handling or electric shock; these data are provided in Table 26. The social development of rats shows some differences between rats raised in isolation and those raised in groups (Baenninger, 1967). Rats raised in isolation show less interest in novel objects and less manipulatory ability than do rats raised in groups (Einon and Morgan, 1976). Circadian rhythms affect corticosterone levels, and there are seasonal differences as well; the levels of corticosterone are lower in summer than in winter (Popova and Naumenko, 1972). Lactation reduces aggressiveness and its physiological consequences in rats (Thomas *et al.*, 1970).

Rats probably possess pheromones: Groups of four have been tested individually for preference of odor of a dominant or subordinate rat, shown in Table 27. Rats housed under various living conditions differ in adrenal function, as summarized in Table 28, and noise alters the effects of various treatments, as noted in Table 29.

TABLE 26 Effects of Early Handling and Shock on Corticosterone Concentrations[a]

Birth	Stimulation[b]	Corticosterone (μg/100 ml plasma) at Age in Days 8	15	21
Handled	Handled	5.15	9.12	12.30
Not	Handled	6.51	8.67	21.50[c]
Shocked	Shocked	6.20	11.10	18.44
Not	Shocked	7.23	9.76	23.76[c]

[a]Adapted from Ader *et al.* (1968). Copyright (1968) by Pergamon Press, Inc.

[b]Rats were or were not handled, were shocked, or were not shocked beginning at birth for 7, 14, or 20 days. Then they were again handled or shocked at 8, 15, or 21 days.

[c]Significant at $p < 0.01$.

TABLE 27 Preferences for Odors in Dominant and Subordinate Rats[a]

Rank of Rat in Its Group of Four	Time in Seconds Spent Smelling Odor of Dominant	Subordinate
I	49	75
II	50	42
III	40	55
IV	45	55

[a]Adapted from Krames *et al.* (1969).

TABLE 28 Corticosterone Plasma of Rats in Differing Population Densities[a]

Treatment or Housing	Rats per Cage	Corticosterone (µg/100 ml plasma)	Blood Taken by
Animal house	20	9.5	Decapitation
Animal house	20	8.5	Aortic puncture
Laboratory	20	27.4	Aortic puncture
Animal house	1	5.8	Decapitation
Animal house	1	5.8	Aortic puncture
Laboratory	1	30.4	Aortic puncture

[a]Adapted from Barrett and Stockham (1963).

Other Rodents

Some 2,000 species of rodents exist, but only a few have been used in the laboratory, and even fewer have been tested for social responses to the environment. The prairie deermouse (*Peromyscus maniculatus bairdii*) has been tested in groups and shows little adrenal response to the usual grouping experiment (see Table 30). However, corticosterone levels will fluctuate (Bronson, 1963). Much of the interest in *Peromyscus* involves the regulation of population size through a mechanism of delayed maturation. The presence of other mice may retard the process by several weeks, as set forth in Table 31.

Young voles (*Microtus*), when raised in groups, are inhibited in their sexual maturation (D. E. Davis, unpublished). Hamsters show some sexual responses to grouping (Vandenberg, 1971), and gerbils have a hormonally effective system of olfactory marks. The presence of male gerbils may cause a female to kill her litter (Ahroon and Fidura, 1976). Also, social inhibition may prevent territorial marking (Nyby *et al.*, 1970). These scattered observations illustrate our lack of information.

TABLE 29 Effects of Noise on Rat Corticosterone[a]

	Corticosterone Concentrations (µg/100 ml plasma)		
	Time (min) after Exposure to 10 min of Noise Stimulus		
Treatment	0	30	60
20 per cage	9.5	15.6	13.8
1 per cage	5.8	7.7	5.9
Handling	6.0	16.6	16.5
Injection, saline	5.7	24.2	26.2
pentobarbitol	4.9	17.0	13.8
histamine	5.8	46.7	55.3
Ether, 1 minute	6.2	38.4	21.7
continuous	6.0	42.4	46.1

[a]Adapted from Barrett and Stockham (1963).

TABLE 30 Species Differences Between House Mice and Deer Mice in Adrenal Response[a]

	Weight of Adrenals (mg)	
Treatment	Deer Mice	House Mice
1 per cage	4.3	3.8
2 per cage	4.9	4.7
4 per cage	4.5	5.3
8 per cage	4.2	5.0
Exposure to fighters[b]	5.3	4.9
Handled[c]	4.8	4.0

[a]Adapted from Bronson (1963).

[b]Of the same species.

[c]Placed alone in a strange cage for 15 minutes each day.

Primates

Abundant evidence indicates that primates respond to stresses through the pituitary-adrenal axis, and a few reports concern social environment. Rhesus monkeys develop a social order. Removal of a subordinate monkey from its group results in a decline of its level of ACTH; conversely, removal of a dominant monkey will bring about a decrease of ACTH in the subordinates (Sassenrath, 1970). In squirrel monkeys, levels of plasma cortisol correlate negatively with rank in a group of five. As might be expected, when a stress is applied, the dominant monkey shows the greatest response (Manogue *et al.*, 1975). The heart rate is high in the top- and bottom-ranking squirrel monkeys in a group (Candland *et al.*, 1970). Increases in 17-ketosteroids, as shown in Table 32, and in atherosclerosis, are products

TABLE 31 Adrenal and Reproductive Weights in Paired and Grouped Deer Mice[a]

Rearing Condition[b]	Adrenals (mg/100 g body wt)	Testes or Ovaries (mg)
Males		
PP	13.9	223.2
PI	15.1	149.8
II	14.5	158.0
IP	14.5	200.9
Females		
PP	17.5	5.1
PI	16.2	5.0
II	16.2	5.0
IP	13.9	5.9

[a]Adapted from Thomas and Terman (1975).

[b]Deer mice were born to pairs (I) or in large populations (P) and then reared either by a bisexual pair (I) or in a group (P).

TABLE 32 Increases in 17-Ketosteroids and Cholesterol in Squirrel Monkeys under Restraint Stress[a]

Treatment	17-Keto (mg/24 hours)	Cholesterol (mg/100 ml serum) Before	After
Psychic stress	0.112	180	228
Box exposure	0.102	181	214
Cage only	0.037	153	160

[a]Adapted from Lang (1967).

of restraint and avoidance testing, and presumably they would be generated by traumatic laboratory procedures. In a preliminary study, Meyer and Bowman (1972) have found that the rearing condition of rhesus monkeys (in isolation, in groups, or in the wild) makes no measurable difference in the response to the stress of restraint. Sackett *et al.* (1973) have shown that the adrenals from isolated rhesus are more active than the adrenals from grouped monkeys. It is suggested that isolation is more of a stress than grouping. The great susceptibility of the adrenal glands to the social environment requires that laboratory managers know what kinds of responses can easily occur.

Rabbits, Dogs, and Cats

I have found no data on responses to the social environment in the laboratory. Perhaps some exist, as the naturally occurring social organization of these mammals has been studied extensively. Individuals show dominance and consequent inhibition of reproduction and increase in mortality--therefore, behavioral and physiological responses should be expected under laboratory conditions.

Birds

The existence of social rank was first recognized in chickens and has been abundantly verified (Schein, 1975). Chickens reared at different densitities (464 cm^2 or 929 cm^2 per bird) have greater left but not right adrenal weights at higher densities (Siegel and Siegel, 1969; Siegel and Latimer, 1975; Siegel, 1976). Pigeons also have social ranks. Although several species have been examined for the relationship of hormones to behavior, data on laboratory conditions do not exist.

Pigs

When gilts are crowded (180 cm of feeder space), they show a dominance order, whereas in less-crowded conditions (360 cm) they do not (Rasmussen *et al.*, 1962). Weight gain also is related to space: at a density of 0.45 m^2, hogs gained 40.05 kg; at 0.90 m^2, they gained 41.85 kg; and at 1.80 m^2, they reached 43.20 kg. The efficiency of food conversion was highest at low densities (Heitman *et al.*, 1961). No difference was apparent in adrenal weights of pigs raised in a space of 1.26, 0.63, or 0.45 m^2 per pig (Addis *et al.*, 1965). Our knowledge of the social environment of pigs is so meager that no conclusons should be drawn (Wiekert, 1971).

NEW SPECIES

The laboratory director is frequently confronted with a request to provide animals belonging to a species not yet maintained in captivity or to maintain some such species for experimental work. For example, occasional reports appear in the literature of lemmings (Dieterich, 1975) or of new deer mice (Forrester, 1975). The director will need some guidance in his attempts to be helpful. For most species, comprehensive information is available in mammalogical and ecological journals on habits, distribution, and populations. Sometimes, data for a closely related species will be instructive. Enough information about mammalian behavior has been obtained to be certain that a hitherto undomesticated species organizes a rank and that reproduction will decrease with crowding. Thus, the manager can concentrate on the details of these relations. The process of using a particular species can be initiated promptly, perhaps with help from those who manage animals in zoos (Crandall, 1964).

CONCLUSIONS

Production of good animals for research and testing requires managers to give careful consideration to animal behavior. The details of housing, handling, pairing, testing, and examining affect the results of an experiment.

The environment includes many features such as noise, duration of light, temperature, and chemicals in the air that affect animal behavior in respect to social organization. More important are the consequences of animals' organization into a social rank. Each individual fights to achieve dominance in a group, but generally only one succeeds. Early infantile experience will make a difference in the behavior of mature animals. The existence of endogenous daily rhythms of activity, and consequent fluctuations of hormones, especially ACTH and corticosterone, must also be taken into account. The social environment has many influences on animal physiology. Dominant animals inhibit reproductive behavior in subordinate animals and reduce a colony's rate of production. Crowding may affect maternal behavior and efficiency of lactation. The response to drugs is generally greater in low-ranking animals. In addition, drug response has a circadian rhythm. Thus, conditions for testing a drug must be rigorously standardized. Similarly, low-ranking animals have less resistance to infection. Depending on the nature of

the resistance mechanism, the result may be positive or negative for the animal. Finally, emotionality or excitability is also affected by the social environment.

A survey of data on the social environment of laboratory animals demonstrates that information has been obtained for testing the repercussions of laboratory animal care. The available data come from studies of population regulation, endocrine function, and psychology of early experience.

RECOMMENDATIONS

1. For laboratories to be accredited, regular research should be conducted on care of animals. This requirement would soon repay any initial trouble by improving animal quality and collaboration among researchers.

2. A set of diagnostic measures should be developed to detect "behavioral disease." For example, size (and perhaps function) of the adrenal glands and the frequency of estrus would be useful signs to monitor.

3. The conditions of rearing and maintenance of all animals should be recorded systematically and the information included with each shipment. The experimenter and the tester of drugs should know about population density, lighting schedule, and shipping conditions, as well as the standard statements of age and strain.

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Discussion

SENTURIA: I am Dr. Senturia from Cleveland State University. I have a question and comment for Dr. Davis in regard to the effect of power failure on the lighting cycle. I would agree that a break in the middle of the lighting cycle has no effect, but if it occurs at onset of light, it will have an effect. Also, power failures will result in missetting of lighting control clocks. Some backup system for resetting clocks must be considered in every installation.

DAVIS: Dr. Senturia has done a number of excellent studies on circadian rhythm, and he is quite correct. After a power failure, clocks must be put back onto the right cycle. There should be some kind of signaling system to warn you that a power failure has occurred.

GOLDSTEIN: I have two questions. One is for Dr. Lindsey. The matter of ammonia production is of great concern to most of us who are involved with both management and housing of animals. How should environmental designers, architects, and engineers in particular be advised to deal effectively with the ammonia problem in the animal environment?

I have a question also for Dr. Davis. Circadian rhythms seem to be referred to constantly, yet seasonal influences are not. I am sure a compelling need exists for establishing a proper relationship between the daily and the seasonal rhythms.

DAVIS: Yes, thank you. Data from Halberg's laboratory and from some of the Russian laboratories indicate a seasonal change in production of corticoids and amplitude of the circadian rhythm from month to month. That would suggest there are seasonal changes. We know that wild animals undergo seasonal changes in the adrenals and so on. My work is concerned with a circannual rhythm. I want to find out if there exists an endogenous annual rhythm that is separate from seasonal changes, which presumably stem from external factors.

I have reason to think this may be the case, because of an experiment in which I sent woodchucks to Australia for 7 years. In 3 years they entrained to the Australian conditions. At our next symposium we should have a lot of information on endogenous animal rhythms, which will also add some more complications.

LINDSEY: In answer to the question about ammonia, there is no easy solution, particularly if we consider that ammonia is just one of many environmental factors altered by the accumulation of waste in cages. Thus, ammonia may serve as an indicator of a variety of environmental changes taking place between each sanitization and replacement of cages.

The related question (one always asked) is, "What kind of cages would you recommend--solid bottom, wire mesh, or some other type?" It is always imperative to keep one's research objective clearly in mind and then try to provide the program that will accomplish the desired result. When housing rodents, one of the most important objectives is to limit transmission of respiratory pathogens because respiratory diseases are extremely common in these species. Cages with solid bottoms probably are superior to suspended wire cages for this reason *if* they are cleaned frequently. We can merely take what little information is available and use it to the best advantage.

*Interactions Between Primary (Cage) and Secondary (Room) Enclosures**

JAMES E. WOODS

Differences between cage microenvironments and laboratory macroenvironments were recognized before the turn of the twentieth century, but the importance of these differences has only been realized fully in recent years. The use of metabolic cages to facilitate experimental protocols was reported as early as 1904 (Henniques and Hansen), but little attention was paid to the environmental quality within these or storage cages until World War II (Reyniers, 1942; Poiley, 1966). As biological, chemical, and radiological research activity intensified, scientists gained a better understanding of the etiology of animal diseases, and they identified the need for improved environmental control within animal cages and laboratories. As a result, more sophisticated cages and environmental control systems and improvements in animal care and handling have developed (Anderson, 1964; Kraft *et al.*, 1964; Reyniers, 1964; Poiley, 1967; Simmons *et al.*, 1968; Yale and Vivec, 1968; McGarrity *et al.*, 1969; Beall *et al.*, 1971; McGarrity and Corriell, 1973).

For the protection of laboratory personnel and laboratory animals, an understanding of the distinction between the primary and secondary environments is required. Weihe (1971) considered the effect of the primary environment on the health of laboratory animals. Using the World Health Organization's (1967) definition of "health," he argued that "health" is the "absence of strain, not only because there is no disease, but also because there is fitness to (adapt to) the environmental conditions." He further reasoned that high adaptability is found in healthy laboratory animals at the prime of life, whereas low adaptability may be a characteristic of animals that are very young or old, inbred, or either purposely or inadvertently diseased. The state of health and comfort of human occupants in the secondary environment has been studied by Rohles and Nevins (1973), who found similar results. Thus, it is necessary to maintain both environments within acceptable limits, although the desired values and allowable tolerances can differ.

Environmental differences between the cage and the laboratory can produce significant variations in experimental results. Mainland and Herrera (1954) suggested that it might be statistically erroneous to assume no differences between cage and laboratory environments and that confounding cage effects with experimental treatments might invalidate an experiment. Since then, several studies have shown that statistically significant differences existed among cage and laboratory dry-bulb temperatures and moisture contents (Weihe, 1965; Simmons *et al.*, 1968; Murakami, 1971; Besch, 1975; Woods *et al.*, 1975a,b); gaseous concentrations (Lillie, 1970; Serrano, 1971; Broderson *et al.*, 1974); and particulate concentrations (McGarrity *et al.*, 1969; Beall *et al.*, 1971; McGarrity and Corriell, 1973; Vessell *et al.*, 1973).

Thus statistical reliability must be considered as a function of physiological and psychological conditions. For instance, a 1.0°C dry-bulb temperature differential may be statistically significant at $p \leq 0.01$, but no

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physiological or psychological consequences may be detected (Weihe, 1971; Besch, 1975; Woods *et al.*, 1975b). Conversely, a 20-ppm difference in ammonia concentrations may not be significant at $p \leq 0.05$, but 70 ppm within the cage could induce respiratory mycoplasmosis or lesions in nasal passages (Broderson *et al.*, 1974). Moreover, the environmental differences may not register as main effects, but their interactions may nonetheless be statistically and physiologically significant. Serrano (1971), for example, compared carbon dioxide and ammonia concentrations in laboratories with those in the microenvironments of three types of protective tops for mouse cages. Significantly higher concentrations were found within the cages. He concluded that using protective covers may reduce transmission of infectious organisms, but that the accompanying increase in such variables as ammonia and carbon dioxide concentrations may have a greater influence on the results of some experiments than would the microbes themselves. Similarly, McJilton and Frank (1973) reported that pulmonary flow resistance increased significantly in guinea pigs exposed to an aerosol mixture of sulfur dioxide and sodium chloride at high relative humidities (80 percent), but that other combinations had insignificant effects.

Although most scientists working with laboratory animals now recognize that cage microenvironmental conditions may differ strikingly from the laboratory macroenvironmental conditions, guidelines for cages and laboratories have yet to be established to take these differences into consideration. The most generally quoted sets of guidelines for selection of thermal conditions are those by Runkle (1964) and a series of standards for breeding, care, and management for certain laboratory animals, first published by ILAR in 1967. Recommended values from these reports, which are summarized in Tables 1 and 2, are for laboratory conditions and do not address the cage conditions. Of further concern is the absence of recommendations for lighting and acoustics.

The Animal Welfare Act (1971) specifies the size of cages that must be employed for certain laboratory animals used in research or teaching, and Table 3 summarizes cage sizes that comply with this legislation. The Occupational Safety and Health Administration (OSHA, 1973) Act Title 29, Part 1910, now requires "enclosed environments" or "isolated cabinets" if suspected carcinogens are used in an experiment. Neither of these regulations specify or recommend cage microenvironmental conditions, although both documents

TABLE 1 Recommended Laboratory Design Conditions[a]

Animal	Weight, kg	$m^3 \cdot min^{-1} \cdot animal^{-1}$	Total Watts/ Animal	Recommended Room Conditions: Dry-Bulb Temperature, °C	Recommended Room Conditions: Percent RH[b]
Mouse	0.022	0.004	0.176	20-24	50-60
Hamster	0.119	0.012	0.732	20-24	40-55
Rat	0.248	0.024	1.260	18-23	45-55
Guinea pig	0.347	0.035	1.641	18-24	45-55
Chicken	up to 3.15	0.115	8.790	--	--
Rabbit	up to 3.6	0.360	9.962	16-24	40-45
Cat	up to 3.6	0.360	9.962	21-24	40-45
Monkey	up to 5.4	0.540	12.599	17-29	40-75
Dog	up to 27.2	2.720	43.950	18-24	45-55

[a]From Runkle (1964). Reproduced with the permission of the *AIA Journal*; 1964, the American Institute of Architects.
[b]Relative humidity.

TABLE 2 Recommended Environmental Conditions[a]

Species	Dry-Bulb Temperature, °C	Relative Humidity, %	Ventilation Rate, Changes$\cdot h^{-1}$
Dogs			
adult	18-29	40-70 (50 recommended)	10-20 (not less than 6)
whelping	27-29		
Mice	21-27	40-70 (50-55 recommended)	10-20
Rabbits	15-21	40-60	10

[a]From ILAR (1967a,b; 1973).

TABLE 3 Space Recommendations for Laboratory Animals in Compliance with the Animal Welfare Act of 1971[a]

Species	Weight	Type of Housing	Square Floor Area/Animal	Height[b]
Mouse	Up to 10 g	Cage	39 cm	12.7 cm
	10-15 g	Cage	52 cm	12.7 cm
	16-25 g	Cage	77 cm	12.7 cm
	Over 25 g	Cage	97 cm	12.7 cm
Rat	Up to 100 g	Cage	110 cm	17.8 cm
	100-200 g	Cage	148 cm	17.8 cm
	201-300 g	Cage	187 cm	17.8 cm
	Over 300 g	Cage	258 cm	17.8 cm
Hamster	Up to 60 g	Cage	64.5 cm	15.2 cm
	60-80 g	Cage	83.9 cm	15.2 cm
	81-100 g	Cage	103.2 cm	15.2 cm
	Over 100 g	Cage	122.6 cm	15.2 cm
Guinea pig	Up to 250 g	Cage	277 cm	17.8 cm
	250-350 g	Cage	374 cm	17.8 cm
	Over 350 g	Cage	652 cm	17.8 cm
Rabbit	Up to 2 kg	Cage	0.14 m	35.6 cm
	2-4 kg	Cage	0.28 m	35.6 cm
	Over 4 kg	Cage	0.37 m	35.6 cm
Cat	Up to 4 kg	Cage	0.28 m	61.0 cm
	Over 4 kg	Cage	0.37 m	61.0 cm
Dog[c]	Up to 15 kg	Pen or run	0.74 m	--
	15-30 kg	Pen or run	1.12 m	--
	Over 30 kg	Pen or run	2.23 m	--
	Up to 15 kg	Cage	0.74 m	81.3 cm
	15-30 kg	Cage	1.12 m	91.4 cm
	Over 30 kg			
Primates[d,e]				
Group 1	Up to 1 kg	Cage	0.15 m	50.8 cm
Group 2	Up to 3 kg	Cage	0.28 m	76.2 cm
Group 3	Up to 15 kg	Cage	0.40 m	76.2 cm
Group 4	Over 15 kg	Cage	0.74 m	91.4 cm
Group 5	Over 25 kg	Cage	2.33 m	213.4 cm
Pigeon[f]	--	Cage	742 cm	--
Chicken	Up to 0.5 kg	Cage	232.3 cm	--
	0.5-2.0 kg	Cage	464.5 cm	--
	2-4 kg	Cage	1,090.4 cm	--
	Over 4 kg	Cage	1,651.7 cm	--

[a]Adapted from *Guide for the Care and Use of Laboratory Animals* (*ILAR*, 1972).
[b]From the resting floor to the cage top.
[c]These recommendations may need to be modified according to the body conformations of particular breeds. As a further general guide, the height of a dog cage should be equal to the height of the dog over the shoulders (at the withers), plus at least 15 cm; the width and depth of the cage should be equal to the length of the dog from the tip of the nose to the base of the tail, plus at least 15 cm.
[d]Primates are grouped according to approximate size with examples of species that may be included in each group: Group 1--marmosets, tupaias, and infants of various species; Group 2--*cebus* and similar species; Group 3--macaques and large African species; Group 4--baboons, monkeys larger than 15 kg, and adult members of brachiating species such as gibbons, spider monkeys, and woolly monkeys; Group 5--great apes.
[e]Only compatible primates can be housed in groups in pens. The pens should be at least 180 cm high, and resting perches, nesting boxes, and escape barriers are necessities.
[f]Sufficient headroom must be provided so that birds can stand erect without crouching.

are based on the recognized distinctions between primary and secondary environments. At present, there are no methods for evaluating primary and secondary enclosures based on desired performance. Therefore, this paper attempts to:

- describe analytical methods by which performance of cage and room systems can be predicted;
- report experimental validation of the analytical methods; and,
- suggest design criteria for primary and secondary enclosures, methods for achieving design criteria, and a method for rating cage performance.

ANALYTICAL METHODS

Relationships between primary and secondary enclosures have been explored via two models: the room-coupled system (RCS) and the supply-coupled system (SCS) (Woods *et al.*, 1975a). The RCS simulates conditions in which the air-exchange rate of the cage depends on air-diffusion patterns of the room and convection currents in the cage. The cage is not directly coupled to an air supply and represents the most common laboratory situation. Three variations of the basic RCS have also been developed: trans-cage RCS, filter-top RCS, and isolation RCS (Woods, 1974). The SCS simulates conditions in which the cage is directly coupled to a conditioned air supply. Communication with the room also may exist, but the air-exchange rate in the cage can be determined explicitly. The second model represents such important laboratory situations as quarantine areas and housing for germfree animals.

Basic Room-Coupled System (RCS)

Heat or mass is transferred primarily by free convection through cage openings (i.e., passive cage) or by forced circulation of room air through the cage (i.e., active cage) (Beall *et al.*, 1971; McGarrity and Corriell, 1973). When this model is used to estimate cage and room mass concentrations, the permeance through the cage's surface material is considered negligible compared with the mass transfer that occurs in cage openings.

Trans-Cage RCS

When multiple cages are placed in a room, the separation required between cages to prevent cross-contamination must be determined. When dealing with the trans-cage coupling problem, three methods of solution may be considered. First, the sizes and performance characteristics of the cages may be selected to ensure sufficient mixing of the air exchanged between the cages and room. Second, the cages may be separated enough to minimize trans-cage coupling without the use of filters. Third, filters may be selected for their ability to reduce cage separation and maintain the concentrations within acceptable limits.

Analysis of this system indicates that accumulations of contaminated air can be expected if care is not taken to protect the cages located "downstream" (Woods, 1974). This problem can occur in horizontal laminar flow cleanrooms (ASHRAE, 1974) and can also have serious effects in conventional systems. Teelman and Weihe (1974) have recommended that animal cage racks be placed at least 2 m apart to avoid confluence of air eddies and resultant cross-contamination. Generally, when proper care is taken in the location of cages, trans-cage coupling is minimized, and the cage mass concentrations and temperatures approximate those of the basic RCS.

Filter-Top Cage RCS

As a protection against microbial contamination of rodents, animals are generally housed in cages with filter tops described by Kraft *et al.* (1964), Poiley (1967), and Simmons *et al.* (1968). These systems differ from the basic RCS in that the air leaving the cage is filtered by the same device that filters the air entering the cage.

Isolated RCS

When an animal must be kept in an isolated cage for quarantine purposes or because exposure to the cage contaminant is considered a potential occupational hazard, to protect the laboratory personnel the animal housing must include a barrier between the primary and secondary environments. This model also may be used when assessing the performance of laboratory hoods or biological safety cabinets.

Supply-Coupled System (SCS)

When protection of the animals from contamination in the laboratory is desired, conditioned air may be supplied directly to the cages before the air enters the laboratory.

Analyses of the energy and mass balances of these systems offer the potential for objective evaluation of cage performance within a laboratory. However, before applications of these methods become practical, values for cage-coupling coefficients, filter efficiencies, cage load-generation rates, and material and geometric properties must be reviewed. In addition, information will be necessary about quality and flow rates of room supply and return air, room load-generation rates, and a description of the air-diffusion patterns within the laboratory.

EXPERIMENTAL EVALUATION

Cages

Basic RCS and SCS have been evaluated by testing a prototype dog cage (Figure 1) designed to represent commercially available cages (Woods *et al.*, 1975b). This cage provided approximately 100 percent of the floor space for beagle-size dogs (i.e., 10 kg) and 64 percent of the floor space for greyhound-size dogs (i.e., 20 kg) specified by

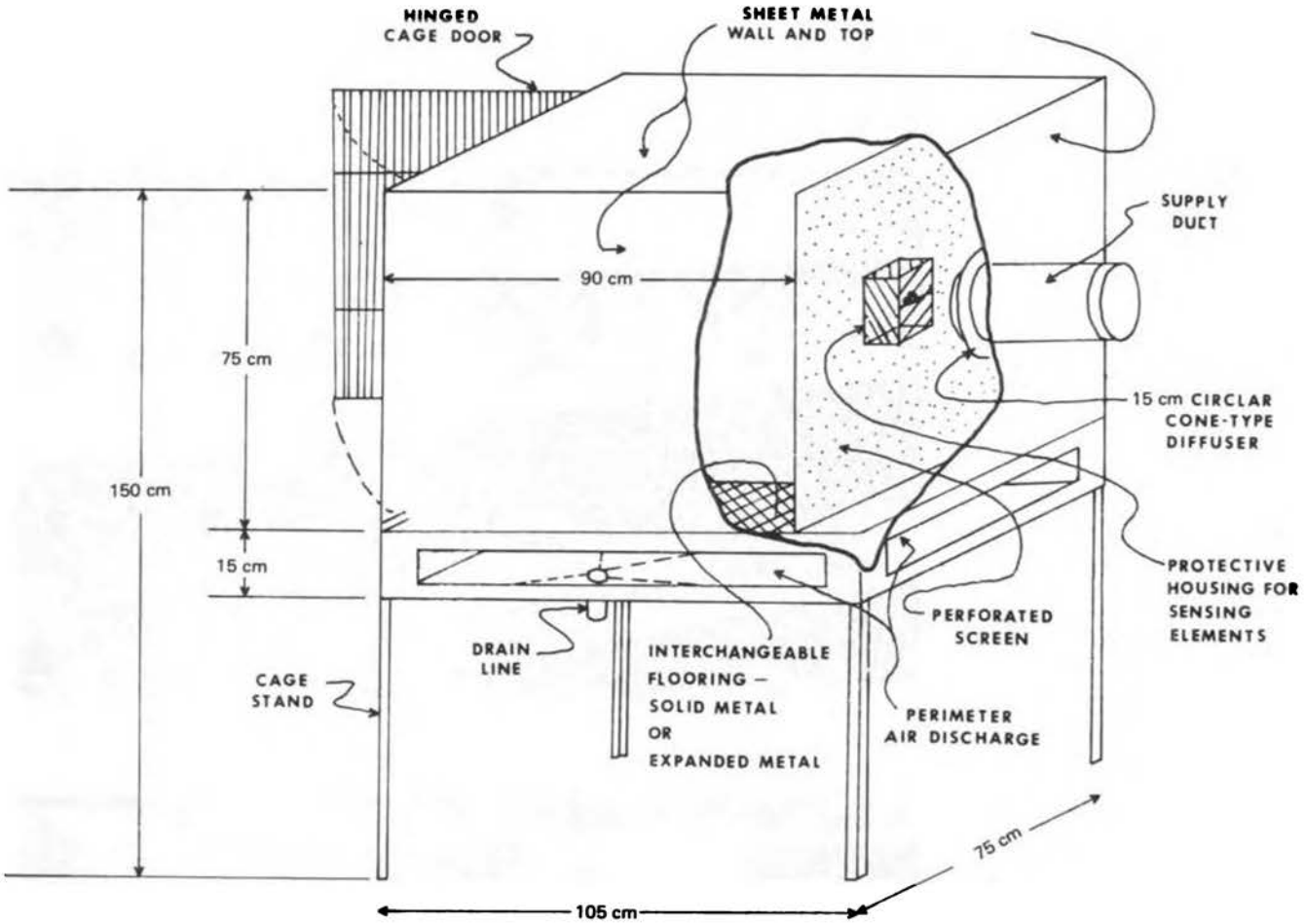


FIGURE 1 Cutaway drawing of prototype dog cage. (From Woods *et al.*, 1975b.)

the Animal Welfare Act (1971). The cage was designed for evaluation with solid and expanded metal flooring. To evaluate the SCS, the supply air duct was connected to a cone-type diffuser in the back of the cage, and the conditioned air was supplied to the cage through the vertical perforated metal panel. The diffuser was capped for the RCS. An expanded metal protective housing was located at the center of the perforated panel to allow cage dry-bulb and dew-point temperatures to be measured when the cage was occupied. The overall heat-transfer coefficient of the cage surface area was determined to be 1.70 $W \cdot m^{-2} \cdot C^{-1}$.

Performances of the filter-top RCS have been determined for standard plastic cages with recommended values of floor areas and cage volumes per animal, as shown in Table 3. The cage dimensions were 0.48 m × 0.27 m × 0.15 m. Tests were conducted with transparent polycarbonate (PC) and translucent polypropylene (PP) cages. All cages contained approximately 2.54 cm of pine shavings for litter material. Cylindrical, perforated metal screens 7.62 cm × 2.54 cm in diameter were located approximately 2.54 cm above the pine shavings at each end of the cages to house the dry-bulb and dew-point temperature sensors. Plastic caulking was applied to prevent air leakage at the sensor locations. The overall heat-transfer coefficient for the PC and PP cages with shavings was measured as 4.30 $W \cdot m^{-2} \cdot C^{-1}$. Commercially available molded, nonwoven polyester filter tops were fitted over the cages but were not caulked in place as reported by Simmons *et al.* (1968). In the PC and PP cages, zinc-plated wire lids were used to contain food pellets and water bottles. The cages, shown in Figures 2 and 3, were placed on the top three shelves of a six-tiered rack located along the sidewall of the environmental chamber.

Load

The dog cage was evaluated while occupied by a 10-kg adult male beagle. The rodent cages were evaluated while occupied by four mature Sprague-Dawley rats (0.423 ± 0.010 kg) and by eight mature golden Syrian (S-D) strain hamsters (0.105 ± 0.002 kg). Each rodent cage contained animals of the same sex, but equal numbers of each sex were tested. All animals were preconditioned for 14 days before the experiment began. During the preconditioning and testing periods, food and water were available *ad libitum*. The photoperiod was maintained at 12 hours of light followed

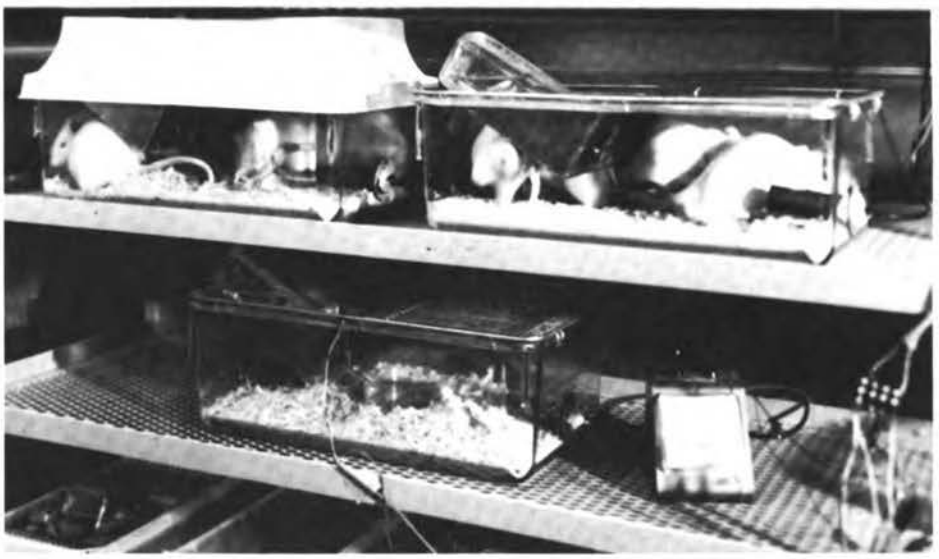
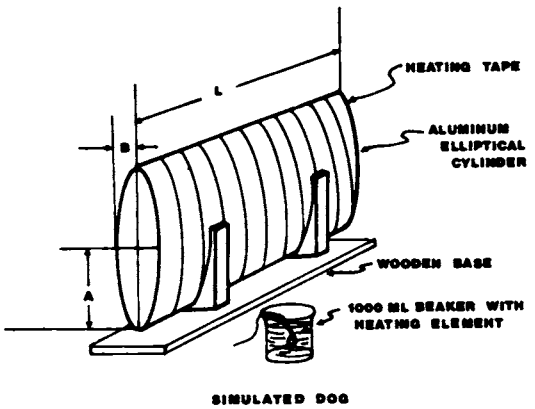


FIGURE 2 Polycarbonate rodent cages. The left-hand cage is covered with a filter top, the right-hand cage is not filtered, and the bottom cage contains simulated rat load. Photo courtesy of J. E. Woods.

FIGURE 3 Polypropylene rodent cages. The left-hand cage is covered with a filter top, the right-hand cage is not filtered, and the bottom cage contains simulated rat load. Photo courtesy of J. E. Woods.

by 12 hours of darkness with the light period between 0600 and 1800 hours. The light was provided by four 160-watt fluorescent lamps located above the perforated ceiling.

Because the loads in each occupied cage could not be determined explicitly, simulated animal loads also were tested. Elliptical aluminum sheet metal cylinders (see Figure 4) were designed to approximate (±5 percent) the volume displacements and surface areas of beagle and greyhound dogs (Woods *et al.*, 1975a). Heating tapes wrapped around the cylinders provided 58 W sensible heat for the simulated beagle (SB) and 108 W for the simulated greyhound (SG). A heating element in



		GREYHOUND		BEAGLE	
		NO BEAKER	BEAKER	NO BEAKER	BEAKER
DIMENSIONS (IN.)	L	24		18	
	A	10		7.8	
	B	2		1.8	
SURFACE AREA, S (FT^2)	DESIGN	8.72	8.72	8.00	8.00
	ACTUAL	8.48	9.08	4.78	8.37
	%ERROR	-.29	3.2	-4.8	7.4
VOLUME V (FT^3)	DESIGN	0.87	0.87	0.38	0.38
	ACTUAL	0.39	0.91	0.37	0.41
	%ERROR	0	3.7	-2.6	8.2

FIGURE 4 Size specifications of simulated dog loads. (From Woods *et al.*, 1975a. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

a 1,000-ml beaker of water provided 19 W latent heat for the SB and 37 W for the SG (Woods and Besch, 1974). Simulated rodent loads were obtained by immersing heating elements in water containers (see Figures 2 and 3). The appropriate sensible:total heat ratios (R) were obtained by heating and evaporating water from beakers modified as described in Table 4 (Gorton *et al.*, 1976). The surface areas and volume displacements were not modeled for rodents.

Environmental Chamber

Cages containing the live and simulated loads were evaluated in a walk-in calorimetric chamber (Woods *et al.*, 1975a). The chamber was controlled at approximately 24°C dry-bulb temperature and 45 percent relative humidity (11°C dew-point temperature) for all tests. The air-exchange rates were controlled at 5, 10, and 15 changes of outside ventilation air per hour. The total airflow was supplied to the chamber through the perforated ceiling during the RCS tests. For the SCS tests, either 50 percent or 100 percent of the supply air was forced through the dog cage before it entered the chamber.

Differential Temperatures

Dry-bulb and dew-point differentials measured during RCS, SCS, and filter-top cage tests are listed in Tables 5 and 6. The differences between simulated and live beagle (LB) values were relatively small compared to the differences in values obtained between simulated and live rodent loads (Besch, 1975; Woods *et al.*, 1975b). Two reasons for the difference in rodent values are apparent. First, the actual sizes of the animals tested were 25-50 percent greater than the sizes assumed in designing the simulated loads. Second, because the rodents used the protective housings for perches, the dry-bulb and dew-point temperatures measured within the cages during live loads were higher than expected. Rats and hamsters tended to crowd the areas of the cages (i.e., those shaded by the food pellets and water bottles) farthest from the direct lighting. This behavior was particularly apparent in animals housed in the unfiltered PC cages. However, the measured differentials are representative of the actual cage microenvironments to which the animals are exposed, and they must be considered in terms of potential thermal stress.

Normalized Cage Characteristics

The normalized dry-bulb temperatures (T) were determined from Equation 1,

$$T = \frac{t_c - t_i}{t_r - t_i} , \qquad (1)$$

TABLE 4 Simulated Rodent Heat and Moisture Loads

Rodent	No. per Cage	$\dot{Q}_t$, W	Sensible Heat Ratios	Beaker Size, ml	Modified Capacity, ml[a]	Water Volume, ml[b]
Rats	4	8.42	0.65	1,000	500	350
Hamsters	8	6.26	0.65	500	350	300

[a]Standard beakers were cut to the maximum volume listed.
[b]Modified beakers were filled to volume listed to obtain the required sensible and latent heat dissipation ratios.

TABLE 5 Dry-Bulb and Dew-Point Temperature Differentials Measured in Dog Cage and Chamber[a]

System	Air-Exchange Rate (changes/h)	Flooring	Dry-Bulb Differential, °K[b,c] Simulated Beagle	Simulated Greyhound	Live Beagle	Dew-Point Differential, °K[b,c] Simulated Beagle	Simulated Greyhound	Live Beagle
RCS	5	Solid	2.50 ± 0.06	3.57 ± 0.07	1.34 ± 0.07	0.70 ± 0.12	0.89 ± 0.23	0.65 ± 0.27
		Expanded	1.97 ± 0.07	3.08 ± 0.02	1.35 ± 0.08	0.42 ± 0.17	0.67 ± 0.24	0.76 ± 0.50
	10	Solid	2.26 ± 0.08	3.61 ± 0.16	1.10 ± 0.03	0.73 ± 0.08	0.70 ± 0.22	0.59 ± 0.29
		Expanded	2.11 ± 0.07	2.95 ± 0.06	1.04 ± 0.08	0.59 ± 0.13	0.61 ± 0.12	0.91 ± 0.19
	15	Solid	1.93 ± 0.10	3.06 ± 0.06	1.07 ± 0.07	0.74 ± 0.11	0.75 ± 0.24	0.64 ± 0.17
		Expanded	1.70 ± 0.12	2.63 ± 0.05	0.96 ± 0.04	0.86 ± 0.23	0.76 ± 0.08	0.90 ± 0.14
50 Percent SCS	5	Solid	0.52 ± 0.09	1.18 ± 0.04	-0.46 ± 0.06	2.14 ± 0.09	2.97 ± 0.16	0.58 ± 0.08
		Expanded	0.77 ± 0.02	1.74 ± 0.05	-0.46 ± 0.08	2.06 ± 0.20	3.78 ± 0.09	0.51 ± 0.14
	10	Solid	-1.03 ± 0.08	-0.22 ± 0.06	-1.26 ± 0.12	0.53 ± 0.09	1.16 ± 0.10	0.28 ± 0.05
		Expanded	-0.27 ± 0.04	0.39 ± 0.07	-1.04 ± 0.09	1.35 ± 0.04	3.51 ± 0.14	0.44 ± 0.10
	15	Solid	-1.06 ± 0.06	-0.74 ± 0.05	-1.22 ± 0.08	0.48 ± 0.04	0.32 ± 0.04	0.29 ± 0.11
		Expanded	-0.79 ± 0.04	-0.37 ± 0.17	-1.17 ± 0.05	0.70 ± 0.10	1.26 ± 0.12	0.44 ± 0.09
100 Percent SCS	5	Solid	-1.84 ± 0.09	-1.39 ± 0.08	-2.13 ± 0.05	0.60 ± 0.13	0.52 ± 0.24	-0.14 ± 0.11
		Expanded	-0.63 ± 0.09	0.66 ± 0.07	-1.89 ± 0.08	1.39 ± 0.07	2.97 ± 0.09	0.51 ± 0.07
	10	Solid	-1.37 ± 0.12	-1.54 ± 0.08	-1.60 ± 0.07	0.35 ± 0.06	0.07 ± 0.30	0.12 ± 0.09
		Expanded	-1.38 ± 0.04	-1.32 ± 0.04	-1.45 ± 0.04	0.36 ± 0.08	-0.15 ± 0.08	0.30 ± 0.05
	15	Solid	-1.08 ± 0.07	-0.96 ± 0.14	-1.18 ± 0.11	0.37 ± 0.11	-0.08 ± 0.11	0.05 ± 0.17
		Expanded	-0.81 ± 0.17	-1.17 ± 0.06	-0.98 ± 0.06	0.12 ± 0.06	-0.20 ± 0.06	0.17 ± 0.08

[a]From Woods *et al.* (1975a). Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.
[b]Temperature shown as mean ± standard error with a sample size of $N = 4$.
[c]Temperature difference of the means ($t_{cage} - t_{chamber}$).

where t_c is cage temperature measured within the protective housings, t_r is the room temperature measured at the chamber exhaust duct, and t_i is the air supply temperature measured in the chamber plenum above the perforated ceiling. When the air-exchange rates in the room were increased (Figure 5), the T values for the dog cage increased in the RCS and decreased in the SCS configurations. The expanded metal flooring in the dog cages resulted in consistently lower T values than the solid metal flooring in the RCS at all values of air-exchange rates in the room. Con-

TABLE 6 Dry-Bulb and Dew-Point Temperature Differentials Measured in Rodent Cages and Chambers[a]

Systems	Air-Exchange Rate, change/h	Cage Material[d]	Dry-Bulb Differential, °K[b,c] Simulated Hamsters	Live Hamsters	Simulated Rats	Live Rats	Dew-Point Differential, °K[b,c] Simulated Hamsters	Live Hamsters	Simulated Rats	Live Rats
No filter	5	PP	1.71 ± 0.02	4.44 ± 0.13	1.69 ± 0.02	5.42 ± 0.13	0.74 ± 0.05	6.53 ± 0.17	0.72 ± 0.04	5.08 ± 0.47
		PC	1.53 ± 0.01	3.68 ± 0.06	1.66 ± 0.02	7.13 ± 0.26	2.00 ± 0.04	7.19 ± 0.07	1.81 ± 0.08	5.39 ± 0.27
	10	PP	1.38 ± 0.01	2.89 ± 0.21	1.62 ± 0.01	6.64 ± 0.46	2.14 ± 0.06	3.46 ± 0.42	1.91 ± 0.11	4.74 ± 0.12
		PC	1.46 ± 0.01	4.76 ± 0.10	1.71 ± 0.01	2.79 ± 0.12	1.71 ± 0.11	1.96 ± 0.03	2.12 ± 0.03	8.03 ± 0.17
	15	PP	1.03 ± 0.01	0.32 ± 0.17	1.40 ± 0.01	0.78 ± 0.04	1.22 ± 0.03	5.43 ± 0.38	0.91 ± 0.08	8.01 ± 0.85
		PC	0.78 ± 0.03	4.26 ± 0.22	1.44 ± 0.02	2.01 ± 0.41	1.33 ± 0.05	3.03 ± 0.31	1.05 ± 0.09	7.39 ± 1.20
Filter	5	PP	2.03 ± 0.01	4.01 ± 0.19	1.90 ± 0.01	5.93 ± 0.17	4.19 ± 0.05	7.51 ± 0.22	5.43 ± 0.07	10.13 ± 0.50
		PC	1.97 ± 0.01	5.02 ± 0.21	2.09 ± 0.01	6.12 ± 0.50	4.64 ± 0.03	6.89 ± 0.12	5.86 ± 0.08	10.44 ± 0.22
	10	PP	1.87 ± 0.01	3.39 ± 0.11	1.83 ± 0.03	6.97 ± 0.34	4.88 ± 0.07	10.79 ± 0.23	7.51 ± 0.09	9.58 ± 0.51
		PC	1.85 ± 0.01	3.72 ± 0.16	2.47 ± 0.04	4.76 ± 0.40	5.38 ± 0.05	4.74 ± 0.15	4.84 ± 0.09	11.13 ± 0.37
	15	PP	1.60 ± 0.01	1.00 ± 0.03	1.96 ± 0.01	3.17 ± 0.20	3.24 ± 0.08	6.93 ± 0.19	3.45 ± 0.12	10.98 ± 0.56
		PC	1.08 ± 0.04	3.58 ± 0.09	2.11 ± 0.01	1.94 ± 0.37	5.32 ± 0.07	7.06 ± 0.09	4.82 ± 0.18	11.84 ± 0.61

[a]Table courtesy of J. E. Woods.
[b]Temperature shown as mean ± standard error with a sample size of $N = 6$.
[c]Temperature difference of the means ($t_{cage} - t_{chamber}$).
[d]PP = translucent polypropylene; PC = transparent polycarbonate.

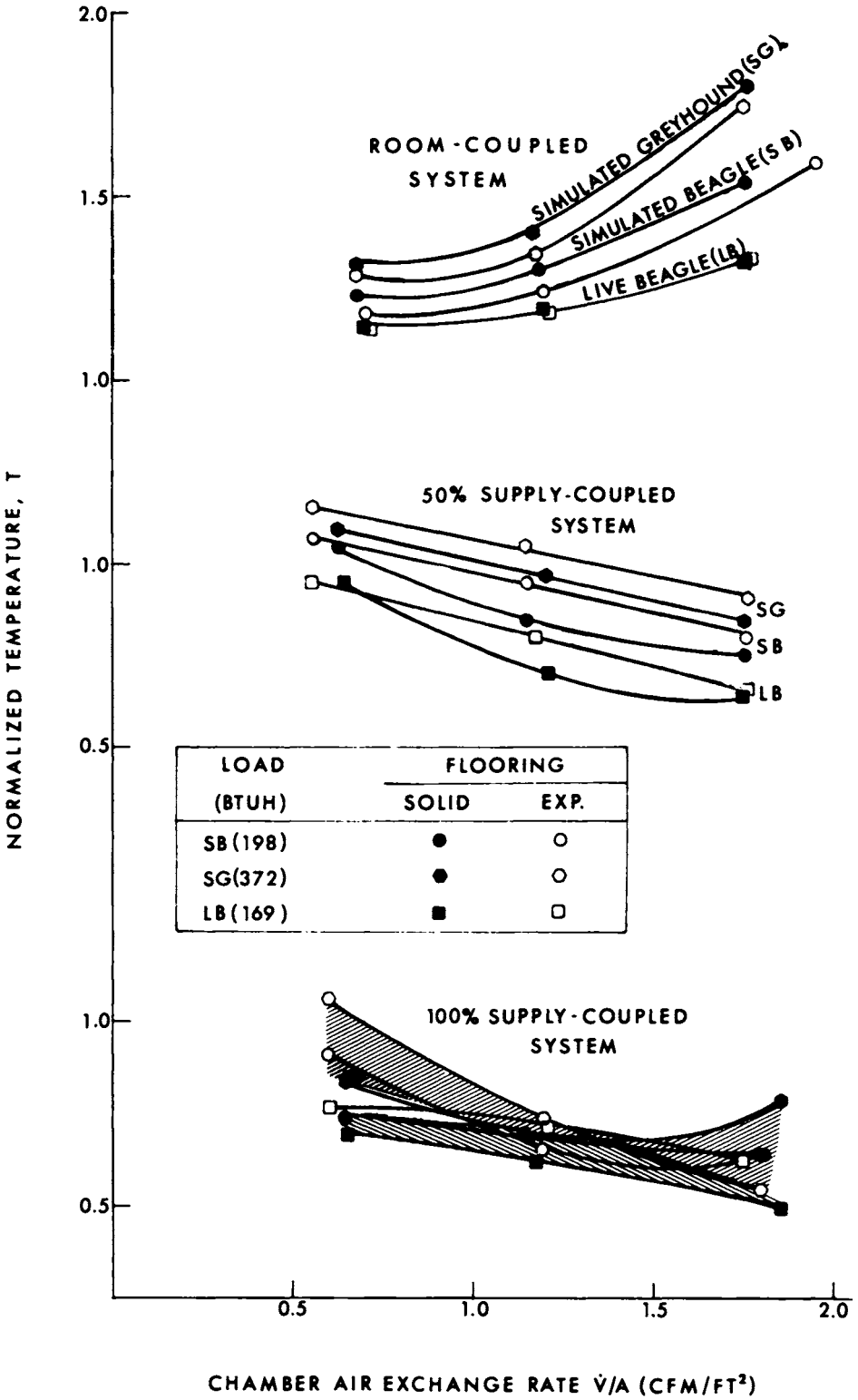


FIGURE 5 Normalized temperatures with dog cage as functions of air-exchange rates in the chamber. (From Woods *et al.*, 1975a. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

versely, consistently higher T values were obtained in cages with expanded metal flooring in the 50 percent SCS. No obvious differences caused by flooring were seen in the 100 percent SCS. These results support the generally accepted practice of holding animals in cages with expanded metal or wire flooring, unless special conditions such as isolation, quarantine, or asepsis require the use of solid flooring.

The performance of the rodent cages with simulated loads was similar to that of the RCS dog cage, as shown in Figures 6 and 7. A general elevation in T can be seen with increasing air-exchange rates in the room and a noticeable dif-

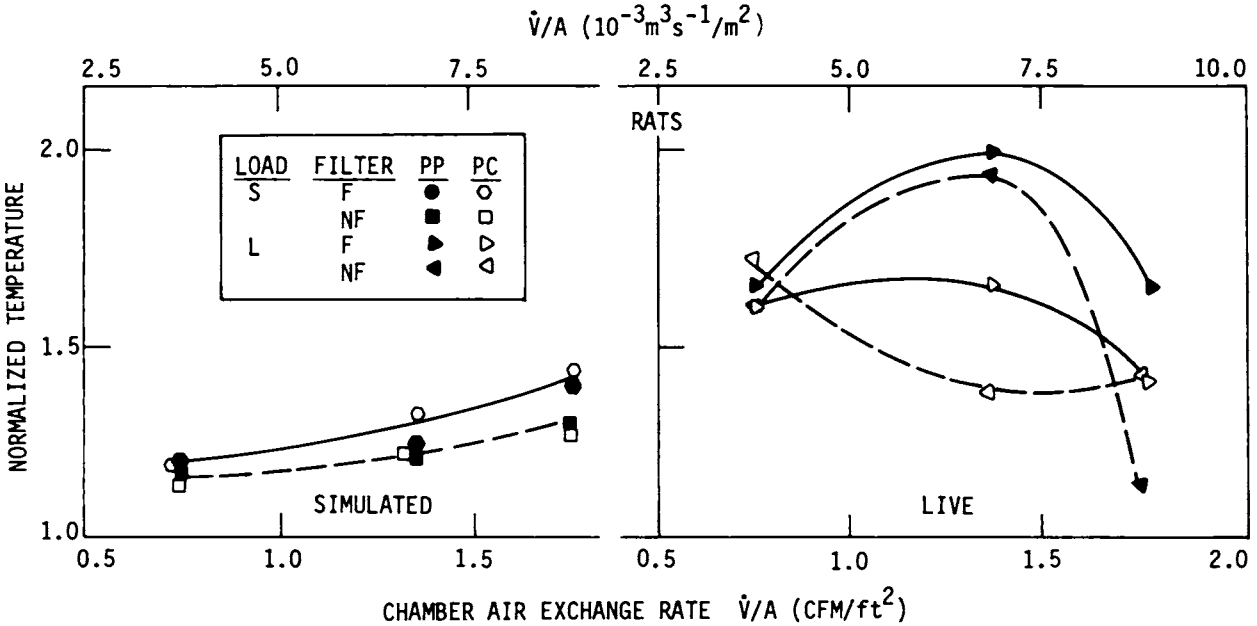


FIGURE 6 Normalized temperatures with rodent cages and rats as functions of chamber air-exchange rates. Diagram courtesy of J. E. Woods.

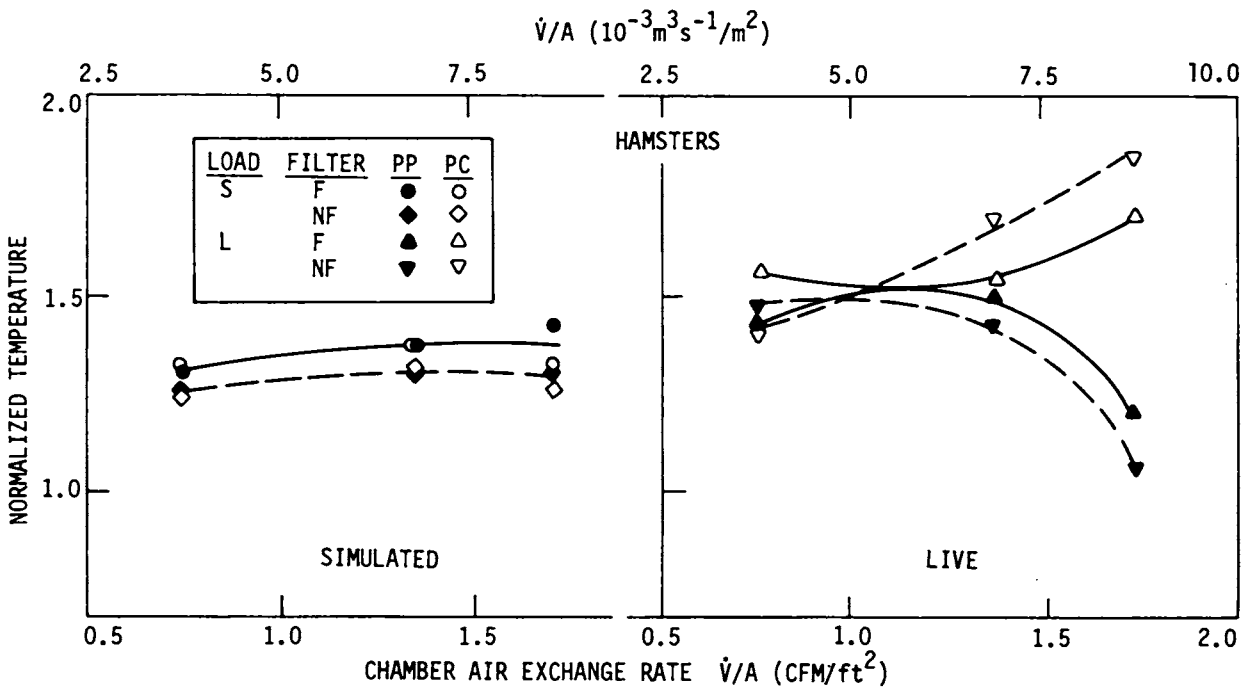


FIGURE 7 Normalized temperatures with rodent cages and hamsters as functions of air-exchange rates. Diagram courtesy of J. E. Woods.

ference can be observed between the T values of filtered and unfiltered cages. With simulated loads, no difference in performance is seen between PC and PP cages. However, when cages were tested with rats (Figure 6), the T values seemed to maximize in the PP cages at $7 \times 10^{-3}m^3 \cdot s^{-1} \cdot m^{-2}$, and a general decrease in T was seen in the PC cages as chamber air-exchange rate increased. Conversely, with hamsters (Figure 7), the T values decreased in the PP cages and increased in the PC cages as the room air-exchange rate was increased. In all cases, except the hamsters in PC cages, the T values were lower in the unfiltered cages.

Unlike the results from the RCS dog cage, the

T values were greater with the live loads than with the simulated loads. This difference was probably caused by the crowding, as previously explained, and it provides further evidence that care must be taken to ensure against extreme heat conditions in rodent cages.

Cage Air-Exchange Rates

Supply-coupling coefficients (β) were fixed at 0.5 and 1.0 for the 50 percent SCS and 100 percent SCS tests, respectively, and became independent system variables. The room-coupling coefficients (α) were not independent, but they could be determined from the analytical methods (Woods *et al.*, 1975a). Based on these values, the rates of cage air-exchange ($\alpha\dot{V}$) for the dog cage and rodent cages are shown in Figures 8, 9, and 10 as functions of the rate of air exchange in the chamber ($\dot{V}/A$).

From Figure 8 it is apparent that expanded metal flooring allowed a greater air-exchange rate than did the solid flooring in the dog cage. That the cage air-exchange rates with the SG were consistently higher than with the SB probably stemmed from the increase in natural convection within the cage caused by the additional heat load and greater cage volume displacement associated with the SG. The increase in $\alpha\dot{V}$ with the live beagle, as compared to the simulated load, probably had to do with the more thorough mixing of air within the cage that was brought about by the animal's movement.

As shown in Figures 9 and 10, when simulated rodent loads were tested, the air-exchange rates in cages without filters were nearly double the values in cages with filters. These values were obtained in a manner similar to that employed to obtain the values for the basic RCS (Woods *et al.*, 1975a), but with the further assumption that the thermal resistance of the filter top was negligible. As in the room-coupled dog cage, the air-exchange rates increased in the rodent cages as the loads were increased from simulated hamsters (SH) to simulated rats (SR). Apparently, with a constant simulated load, PC cages had smaller airflow rates than PP cages. As was detected in evaluating the T values, the cage performances with the live beagle differed from those with the live rodents. In the latter case, the air-exchange rates in the cages with live rodents appeared to be smaller at the two lowest of the room airflow rates than cages with simulated loads. This reduction was probably caused by crowding in the cages, which would result in the air stratifications indicated by the elevated temperatures measured in the protective housing.

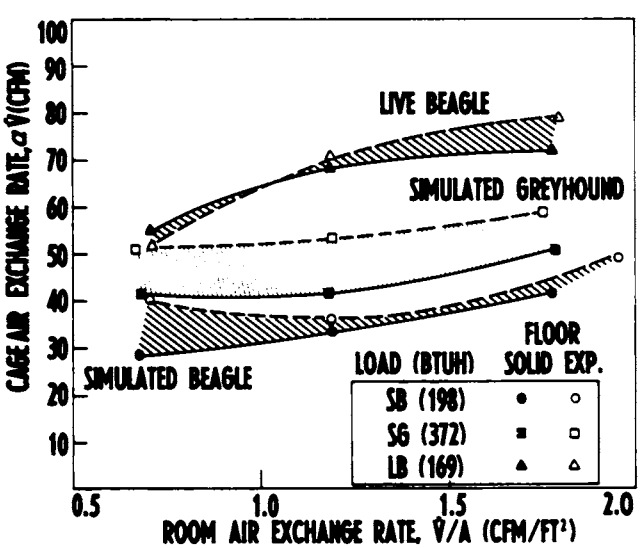


FIGURE 8 Cage air-exchange rates in RCS, as determined from cage characteristics and sensible dog loads as functions of room air-exchange rates. (From Woods, 1975. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

Prediction of Cage Mass Concentration

The analytical models developed for room-coupled and supply-coupled systems indicate that steady-state mass concentrations can be predicted inside cages when certain characteristics of cage performance (i.e., α, β, T), mass-generation rates, and the desired room air concentration are known.

To assess the ability to predict cage performances, humidity ratios (i.e., mass of water vapor per unit of dry air) were predicted for the cage microenvironment based on α values determined from the sensible heat-transfer analysis (i.e., based solely on temperature differences).

The filters on the rodent cages were found to be approximately 78 percent effective (i.e., $\varepsilon = 0.78$) in preventing the transfer of water vapor across the media. No weight increase was detected in the filters when measured with an analytical balance before and after each test (i.e., 8 hours). Therefore, the filtration mechanism must have acted more as a sieve than an adsorption device. When adsorption is not a factor, concentrations of other gases and vapors with similar molecular weights and diffusivity constants (i.e., ammonia) would be expected to increase by approximately the same percentage as the increases found with water vapor. This phenomenon tends to support work by others who have reported increased cage concentrations of gases (Serrano, 1971; Broderson *et al.*, 1974).

Calculated values of cage humidity ratios were compared to values determined from the dew-point temperature measured in the protective housings inside the cages. Percent errors were then determined; they are summarized in Table 7 as means ± standard deviations. The values reported for the basic RCS, 50 percent SCS, and 100 percent SCS are exclusive of 3 data points, whereas the values for the rodent cages are exclusive of 6 points, because each point exceeded the 95 percent confidence interval for all data in the sets.

Table 7 indicates that the percent errors were not significant ($p \leq 0.05$) and that the standard deviations were of the same magnitude as the means in all tests conducted in the dog cage and in the unfiltered rodent cages tested with simulated

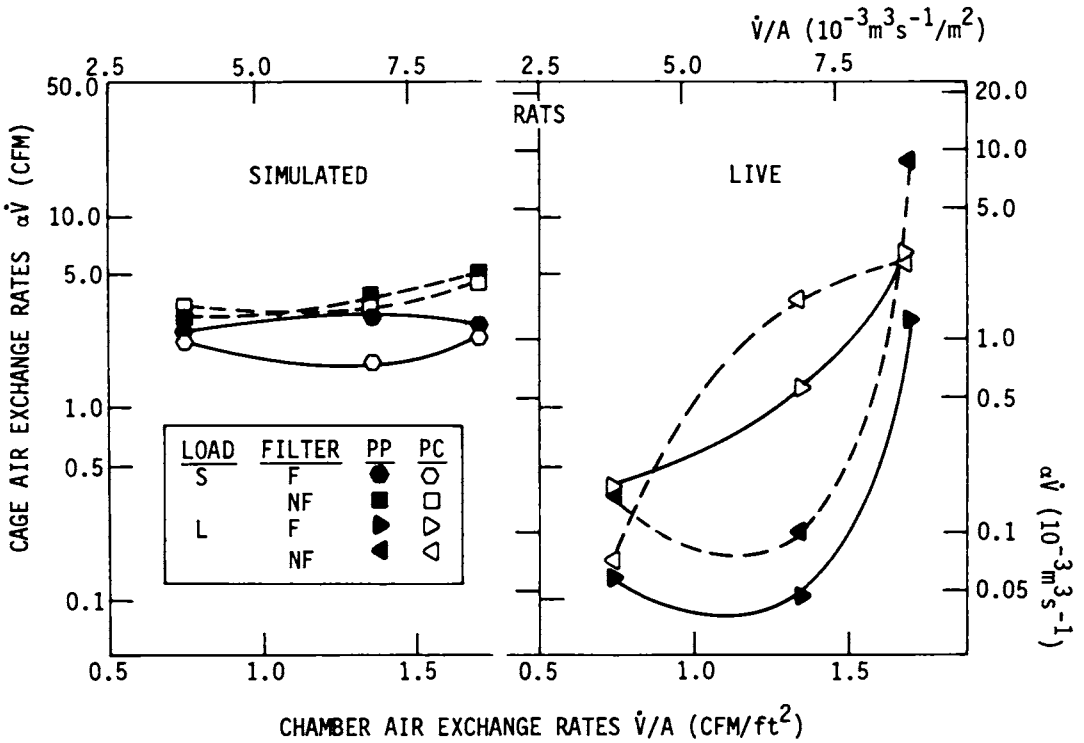


FIGURE 9 Cage air-exchange rates, as determined from cage characteristics and sensible rat loads as functions of chamber air-exchange rates. Diagram courtesy of J. E. Woods.

loads. However, the standard deviations were much larger than the means when unfiltered cages were tested with live rodents and in all tests conducted with filters. The greater magnitudes of the standard deviations in rodent cage tests are thought to be caused by the reduced cage size and the percentages of total sensible heat generated in the rodent cages compared to the percentages found in the dog cages. Tests with simulated hamsters and rats in the filtered cages resulted in percent errors of 5.73 ± 17.59 and -0.74 ± 11.38, respectively. Volume displacement, crowding, activity and perching on protective housings by the live rodents probably account for the additional variance seen in these tests.

An uncertainty analysis indicated that the limit of error for humidity ratios determined from the dew-point temperatures was $\pm 6.7 \times 10^{-5}$ kg H_2O/kg air. For an average humidity ratio of approximately 9.2×10^{-3} kg H_2O/kg air (12.8°C dew-point temperature), the limit of error was 0.73 when expressed as a percent error. Therefore, it is reasonable to assume that the cage mass concentrations were predicted with sufficient certainty to include heat- and mass-transfer characteristics in the criteria required for further cage selections.

Effect of Room-Air Diffusion Patterns

Unless special conditions dictate the use of an air-distributing ceiling, such as that used in the calorimetric chamber or in a laminar flow cleanroom, air normally will be supplied to the laboratory through grilles, diffusers, or registers. A method to evaluate the performance of these systems is known as the Air Diffusion Performance Index (ADPI) (Nevins and Miller, 1972). To test the effect of room-air diffusion patterns on cage performance, two common types of terminal air devices were tested in the Air Diffusion Laboratory at Kansas State University: a high sidewall grille (0.61 × 0.15 m) and circular ceiling diffusers (0.15 m diam.). The high sidewall grille (HSG) was adjusted to make the vanes straight. The two ceiling diffusers (CD) were adjusted to an intermediate position (one-half the number of turns from horizontal to vertical flow), and the volume dampers were adjusted to provide the same flow from each CD (±5 percent). Two prototype dog cages, loaded

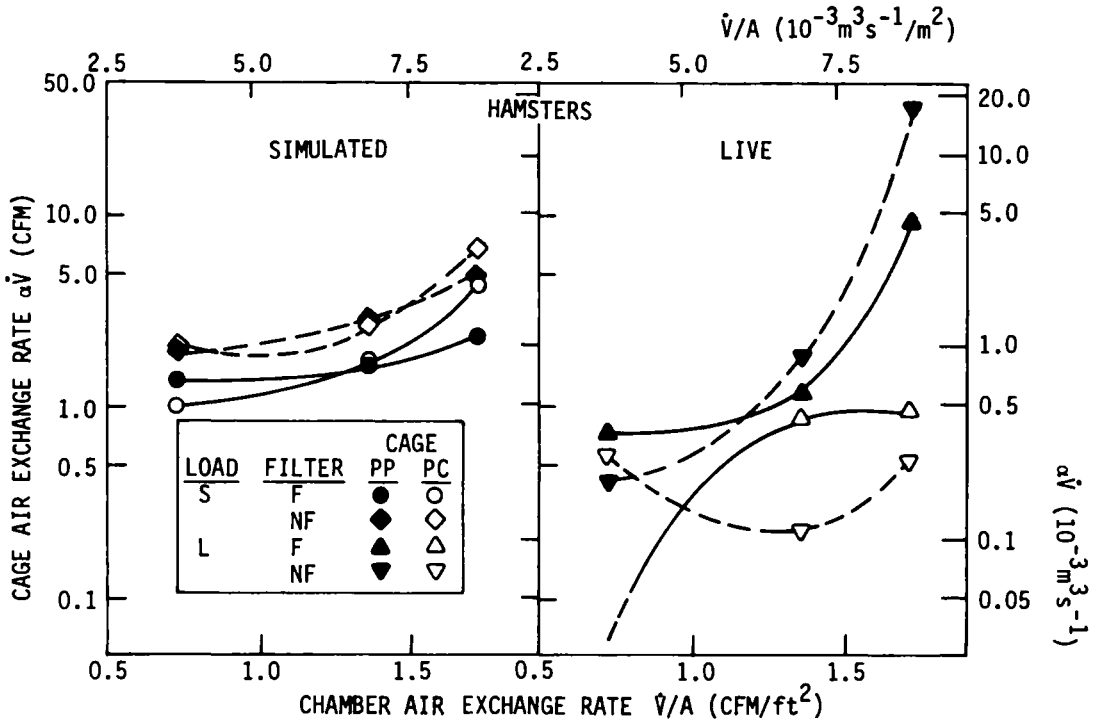


FIGURE 10 Cage air-exchange rates, as determined from cage characteristics and sensible hamster loads as functions of chamber air-exchange rates. Diagram courtesy of J. E. Woods.

with simulated beagles, were placed in the laboratory, as shown in Figures 11 and 12. The total system sensible load was maintained at approximately 63 $W \cdot m^{-2}$ and was tested at four airflow rates. The cages in the room were tested as room-coupled systems.

Results of these tests for the HSG and CD systems are presented in Figures 13 and 14. The values for cage air-exchange rates ($\alpha\dot{V}$) were determined for each cage in the laboratory, as summarized in Equation 2:

$$\alpha\dot{V} = \frac{\phi\,\dot{Q}_s}{1.08\,(t_c - t_r)} - \frac{UA_s}{1.08}, \qquad (2)$$

where $\phi\,\dot{Q}_s$ is the sensible heat load generated within the cage, UA_s is the heat-transfer rate

TABLE 7 Percent Errors in Predicted (w_{cp}) and Measured (w_{cm}) Cage Humidity Ratios[a]

	Dog Cages[b]						Rodent Cages[c]			
	Basic RCS		50 Percent SCS		100 Percent SCS		Filtered		Nonfiltered	
Load	N	M ± SD[d]	N	M ± SD[d]	N	M ± SD[d]	N	M ± SD[d]	N	M ± SD[d]
Simulated	11	0.42 ± 1.43	11	-6.32 ± 5.39	11	-2.45 ± 3.41	12	2.49 ± 15.55	12	-6.31 ± 7.80
Live	6	-1.98 ± 2.99	6	-0.93 ± 1.33	6	-1.11 ± 1.39	9	12.31 ± 40.01	9	-5.36 ± 28.57
Total	17	-0.86 ± 1.77	17	-4.76 ± 4.73	17	-1.98 ± 2.88	17	6.70 ± 28.25	21	-5.90 ± 18.98

[a] $\Delta\% = \frac{w_{cp} - w_{cm}}{w_{cm}} \times 100\%$.

[b] From Woods *et al.* (1975a). Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.

[c] Courtesy of J. E. Woods.

[d] No percent errors were significantly different from zero at $p \leq 0.05$.

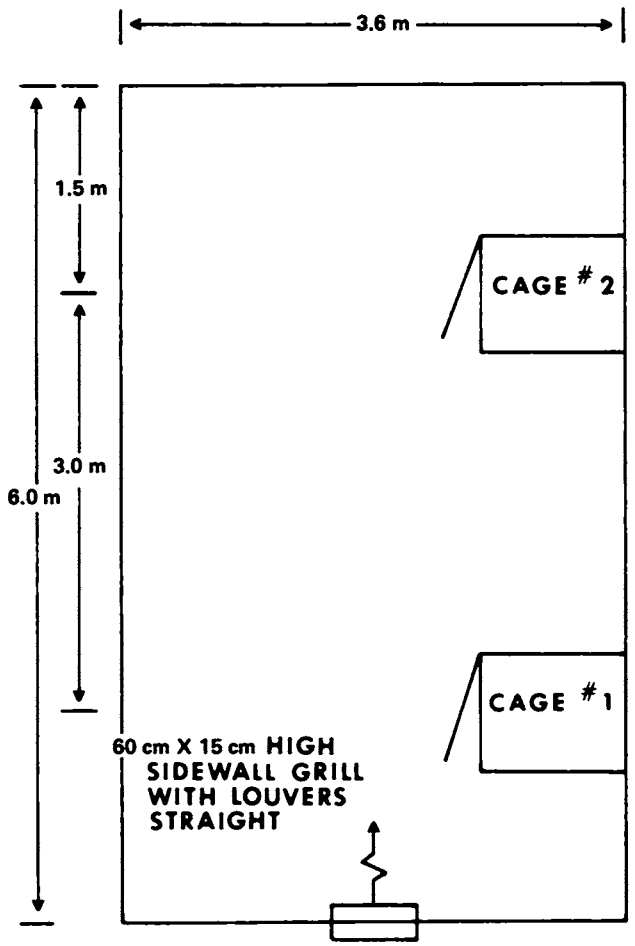


FIGURE 11 Cage locations for ADPI evaluations in laboratory with high sidewall grille. (From Woods, 1975. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

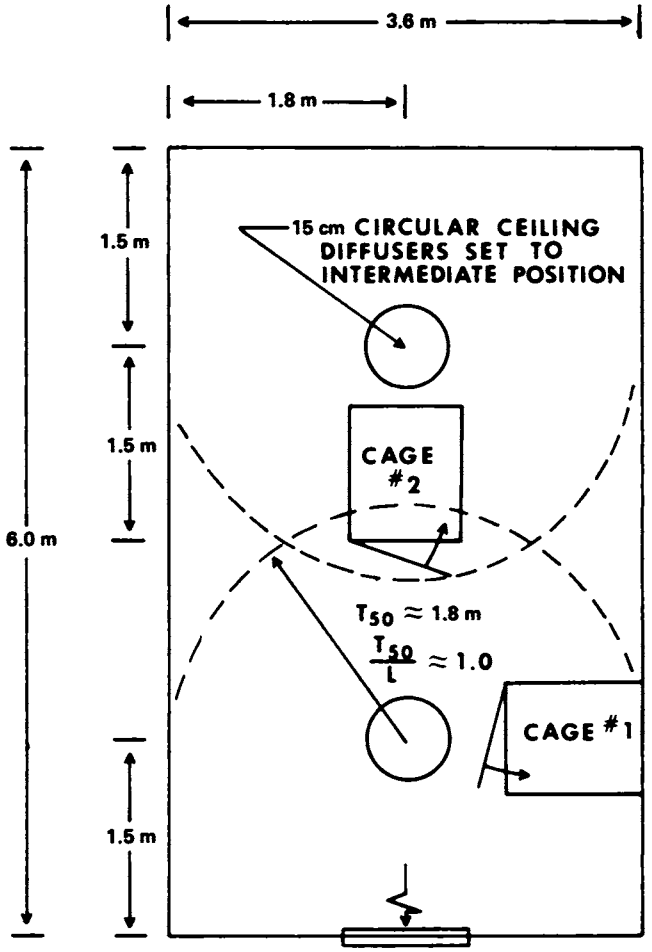


FIGURE 12 Cage locations for ADPI evaluations in laboratory with circular ceiling diffusers. (From Woods, 1975. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

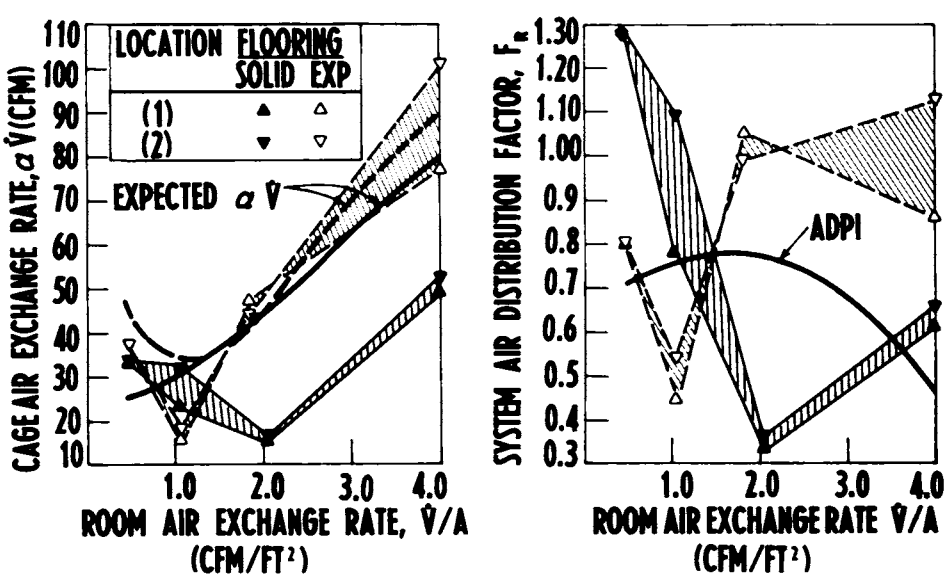


FIGURE 13 Cage air-exchange rates and system air-distribution factors and functions of room air diffusion by the high sidewall grille. (From Woods, 1975. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

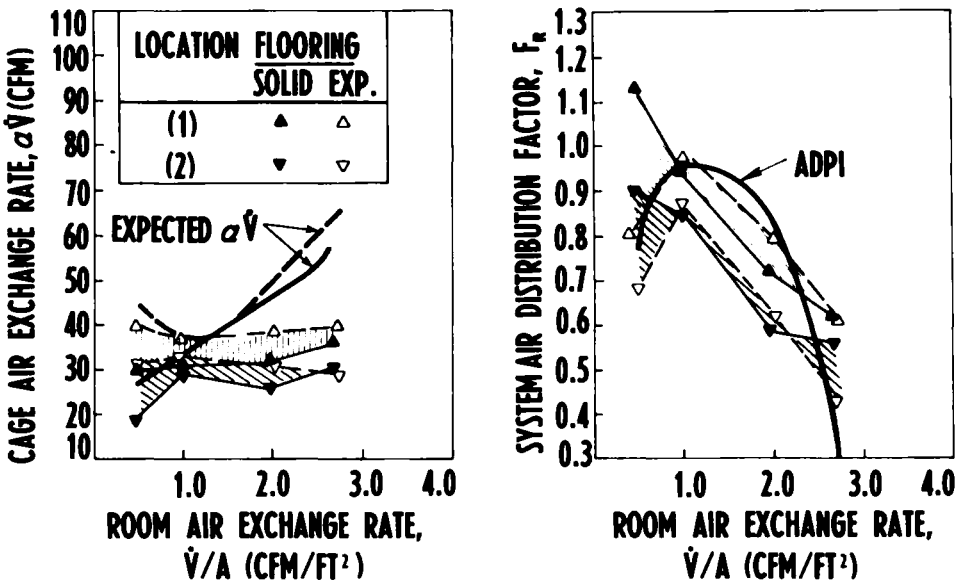


FIGURE 14 Cage air-exchange rates and system air-diffusion factors as functions of room air diffusion by circular ceiling diffusers. (From Woods, 1975. Reprinted by permission of the American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc., from *ASHRAE Transactions*, copyright 1975.)

through the cage surface areas, and $(t_c - t_r)$ is the difference between the cage and room temperatures. The ratio of $\alpha\dot{V}$ in each system to the expected cage air-exchange rate determined in the calorimetric chamber has been identified as the "system air distribution factor," F_R (Woods, 1975). Miller and Nevins (1969) showed that the ADPI in a room with an air-distributing ceiling was independent of the room air exchange rate. The independence of the ADPI and room air-exchange rate also was verified in the calorimetric chamber (Woods, 1975).

Relationships between $\alpha\dot{V}$ and $\dot{V}/A$, and F_R and $\dot{V}/A$ indicate that differences in cage performances stemmed from interactions among type of air terminal device, cage location, and type of flooring. In the HSG system, the cage closer to the supply and return devices had slightly lower air-exchange rates and F_R values. In the CD system, the cage located with its cage door at the approximate confluence of the airstreams from the two diffusers (Figure 14, location 2) had slightly lower values of $\alpha\dot{V}$ and F_R than the cage served by one diffuser. The type of flooring affected the cage performance more than cage location in the HSG system. Conversely, the cage location had more effect on cage performance in the CD system than did the flooring type.

Figures 13 and 14 also show the ADPI values as functions of $\dot{V}/A$ for the HSG and CD systems, respectively. When maximum values of ADPI are compared with the corresponding F_R values, it may be seen that in HSG systems, air-exchange rates in cages with expanded metal flooring are nearly identical to those determined in the calorimetric chamber (i.e., $F_R \approx 1.0$). In CD systems with maximum ADPI, cages with either flooring in location 1 (Figure 14) also had air-exchange rates similar to those measured in the chamber (i.e., $F_R \approx 0.9$-1.0).

Miller and Nash (1971) have demonstrated that relationships exist between the ADPI and a parameter (T_v/L) in which T is identified as the "throw" of an isothermal air jet to a distance where the terminal velocity is $v = 0.25$ m·s^{-1} and L is a characteristic room length. Woods (1975) also showed that for a particular system a linear relationship can be established between T_v/L and $\dot{V}/A$. To maximize ADPI, values of $T_{0.25}/L$ should be designed at 1.5-2.0 for HSG systems and at 1.0-1.5 for CD systems. Therefore, cage air-exchange rates can be predicted in laboratories designed to maximize ADPI, if cage performance and data taken under standard conditions can be obtained.

SUGGESTED DESIGN CRITERIA

The systems described above include variables associated with the cage microenviromnent, the laboratory, and the cage-laboratory coupling effects. Therefore, it is recommended that design criteria be specified separately for the primary (cage) and secondary (room) environments and that cages be selected to meet both sets of criteria. The primary values will vary according to animal species and nature of the experiment, but sufficient information is available in the literature to select most of the required criteria.

Upper critical temperatures (Weihe, 1971) may be chosen to represent maximum dry-bulb temperatures at which the cage is to be maintained to ensure maintenance of thermal neutrality. However, the effects of relative humidity (RH) must also be considered. Values of about 50 percent RH have been suggested for maintaining thermal neutrality and maximizing disease suppression (Baetjer, 1968; Weihe, 1971; Green, 1974). Laboratory thermal conditions are normally specified at 24°C and 50 percent RH for thermal comfort of the human occupants (ASHRAE, 1972).

Design criteria for gaseous and particulate concentrations are more difficult to identify.

TABLE 8 Sample Recommended Design Criteria for Laboratories with Caged Dogs[a]

Environmental Variable	Laboratory Condition	Cage Condition
Dry-bulb temperature	21-24°C	24-27°C
Relative humidity	40-60 percent	40-60 percent
Ammonia concentration[b]	<25 ppm (TLV)	<25 ppm
Dusts, fumes, mists[c]	<15 $mg \cdot m^{-3}$ (TLV)	<15 $mg \cdot m^{-3}$
Viable particulate[d]	<18 $CFU \cdot m^{-3}$	18 $CFU \cdot m^{-3}$

[a]From Woods (1974).
[b]TLV; other gases to be specified as necessary.
[c]TLV for moderately toxic dusts.
[d]$CFU \cdot m^{-3}$ (Colony Forming Units/m^3 of air). More restrictive limits may be necessary, depending on the type of microbes.

Values for the macroenvironment are normally chosen to comply with threshold limit values (TLV), which are annually reviewed and published by the American Conference of Governmental Industrial Hygienists. Cage concentrations are seldom specified as design values. However, since most TLV's are based on studies with animal models, it may be possible to develop design specifications by weighing the TLV's. An example of design criteria is set forth in Table 8.

CAGE SELECTION TO MEET DESIGN CRITERIA

Once the criteria for the primary and secondary environments have been established, the required cage performance characteristics (i.e., α, β, T) can be determined from the equations associated with the appropriate systems. From these characteristics, the minimum air-exchange rate required to maintain the design conditions can be established. Then, using the room air-exchange rate associated with the design ADPI, the expected cage air-exchange rates for certain cage designs can be determined. Comparison of these cage air-exchange rates should yield an objective design procedure to match cage selection to the specified design criteria.

For example, minimum values of $\alpha\dot{V}$ to maintain cage dry-bulb and humidity ratio within the thermal neutral zone for dogs (i.e., 27°C and 50 percent RH) are shown in Figure 15. These minimum $\alpha\dot{V}$ values were determined assuming two-thirds of the heat and all the water vapor generated in the cage would be dissipated by the cage airflow. Superimposed on the minimum values are the cage air-exchange rates determined from the prototype RCS dog cage. Figure 15 indicates that the prototype cage would exceed the minimum cage air-exchange rate for a beagle at any desired room air-exchange rate. Further, the only combination that would be acceptable for a greyhound-size dog would be the expanded metal flooring and a minimum room air-exchange rate of 32 $m^3 \cdot h^{-1} \cdot m^{-2}$.

Methods of achieving desired particulate control are commercially available, but filtration characteristics are not readily obtainable. Data on filter efficiency are available for laminar flow systems that incorporate high-efficiency particulate air (HEPA) filters, but no information has been published about the efficiency of filter tops often used on rodent cages (Poiley, 1967; Beall *et al.*, 1971; McGarrity and Corriell, 1973).

Interaction of factors associated with conventional control methods must also be considered. For instance, if a filter is selected for its particulate efficiency based on weight, size, or number analysis, its heat-, moisture-, and gas-transfer characteristics should also be reviewed.

A SUGGESTED METHOD FOR RATING CAGES

To minimize sources of variance, basic cage performance should be evaluated in a calibrated chamber similar to that used in the tests reported in this paper. Specifications for such a facility could be patterned after the American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) Standard 16-69 (1969).

The cage should be located near the geometric center of the test chamber. To study potentially stressful limits and not harm live animals and allow more freedom in locating sensing elements within the cage, tests should be conducted with simulated loads. However, results achieved with simulated loads must be verified sufficiently with actual loads to establish confidence in the results. To reduce measurement errors with simulated and live loads, the chamber should be calibrated and adjusted so that the load generated within the cage is the dominant load of the chamber.

Cage coupling coefficients should be reported in terms of a variable in the room such as $\dot{V}/A$. Therefore, cages should be evaluated at several values of the room characteristics. Assessments of cage performance also should include information pertaining to variances of values and gradients that develop within the cage (Woods, 1974). A recommended cage classification is set forth in Table 9.

Cage performance and evaluation standards should be developed by organizations familiar with establishing national standards. Therefore, professional groups such as the American Association for Laboratory Animal Science (AALAS) and

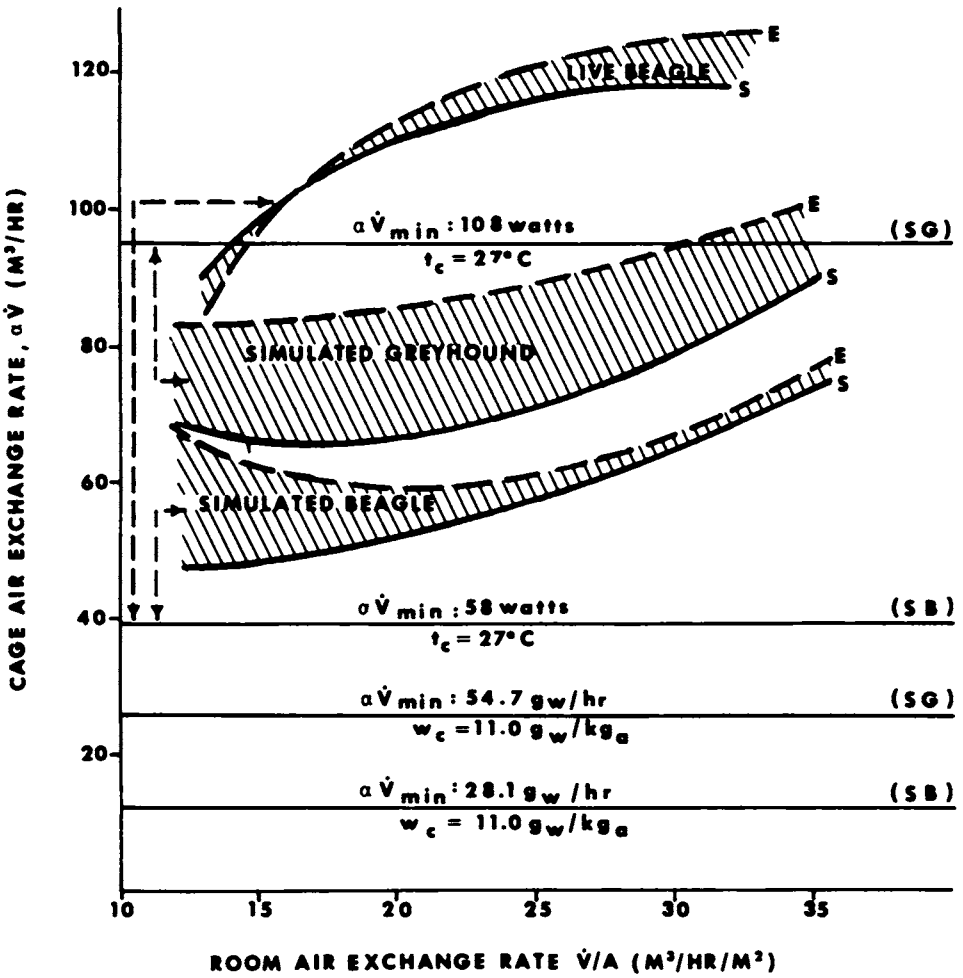


FIGURE 15 Comparison of cage air-exchange rates in prototype dog cage with minimum values required to maintain microenvironment below the upper critical temperature. Diagram courtesy of J. E. Woods.

ASHRAE could be asked to consider writing and promulgating the proposed standards. By this method, members of the participating organizations could adopt the standards through voluntary compliance. Cage manufacturers or independent testing laboratories could evaluate cage performance in accordance with these standards, and the findings could be published. By following these procedures, physiologically neutral environmental conditions to which laboratory animals are exposed could be maintained more confidently and assurance of more uniform environmental conditions between cages and between laboratories could be obtained.

TABLE 9 A Proposed Cage Classification System[a]

Classification	Description
1	Meets thermal requirements of maintaining cage dry-bulb and humidity conditions below the specified upper critical temperature; also meets required husbandry conditions.
2	Meets Class 1 conditions and maintains gaseous and particulate concentrations below specified conditions.
3	Meets Class 2 conditions and provides germfree microenvironment.
4	Meets Class 1 conditions and provides special conditions as specified.

[a]From Woods (1974).

SUMMARY

Analytical and experimental data have indicated that:

- Significantly elevated dry-bulb and dew-point temperatures are common in cages, especially filter-top cages.
- Crowding of rodents resulted in much higher cage temperatures than predicted or than measured when cages were tested with simulated loads.
- Better air circulation was obtained in room-coupled dog cages with expanded metal rather than solid flooring, a finding consistent with recommended animal care and management procedures.
- Air exchange was better controlled in supply-coupled dog cages with solid flooring, a finding consistent with good control of contamination.

- Air-exchange rates in filter-top cages were approximately half those of unfiltered rodent cages.
- Differences in rodent cage materials evidently affect the heat-transfer and air-exchange rates between primary and secondary enclosures.
- Filters on rodent cages act as sieves of approximately 78 percent effectiveness in preventing transmission of water vapor; they probably have a similar effectiveness in preventing transmission of other gases and vapors with similar molecular weights and diffusivity constants (i.e., ammonia).
- When room-coupled dog cages are located in a room with air supplied by high sidewall grilles, the type of cage flooring has greater effect than location on cage performance; however, if air is supplied by ceiling diffusers, the location is more influential than the type of flooring.
- Because interactive effects between primary and secondary enclosures can be predicted and controlled, specifications for laboratory animal housing should include criteria for the cage and for the laboratory.
- Professional organizations such as AALAS and ASHRAE should be asked to develop voluntary standards for cage performance and evaluation.

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Engineering Objectives for Laboratory Animal Housing

ARNOLD L. WINDMAN and ARTHUR L. ZIGAS

Not only must mechanical and electrical systems serving laboratory animal housing maintain correct environmental conditions in an absolutely reliable way for the animals, but they also must assure a satisfactory work environment for the research staff 24 hours a day, 365 days a year. In addition, they must protect the animals from the possible spread of toxins and pathogens. Proper safety and reliability depend not only on the initial incorporation of proper systems and equipment into the facility, but also on the proper and continuous maintenance of the systems once they have been installed.

ELECTRICAL SYSTEMS

Electric Power Supply

Reliable electric power service is essential. National Fire Protection Association Standard 76 A (1973) should be used when designing electrical power systems. Isolated power centers may not be required for animal operating rooms, whereas uninterrupted power supplies may not be required for instrumentation and operating rooms. Power supply for computer terminals in an animal housing facility should have the same degree of reliability as the power supply for the main computer to which they connect. Since there is no stringent requirement for quick, emergency power responses, either diesel or gas turbine-driven generators may be used for this service, if there is no uninterrupted power supply system.

Lighting

Fluorescent lighting supplemented by incandescent is recommended for good rendition of color, reduced heat, and conservation of energy. Intensity of light should be task-oriented: Brightness should be tailored to animal storage areas, feed storage areas, cage-washing areas, and surgical areas. Preferred luminaires are surface-mounted, water-tight, and caulked. Multiple-level switches (controlling the number of lights energized) and time switches are recommended. When feasible, dimming devices useful for adjustment and conservation should be installed. To keep noise levels as low as possible, electric contactors should be located away from animals and people. Time switching of lights, with override adjustments to allow for circadian rhythms, will probably be required. Time switches should have emergency-spring drives to ensure continuity.

Instrumentation

To reduce electromagnetic interference, instrumentation and associated wiring should be located at least 1.5 m away from fluorescent light dimmers and electric motors. Automatic voltage regulators and isolation transformers for instruments must be provided and three-wire grounded cords always must be used.

Safety Checks

All electrical outlets, exposed wiring, and equipment located within areas subject to wash-down should be waterproof. Receptacles with twist locks are desirable for prevention of inadvertent disconnection, but compatibility with equipment already in place should be investigated. Outlets should be mounted as high as is practical. To prevent transmission of organisms via air, all electrical conduits in isolation, quarantine, and

infectious disease units should be caulked or sealed.

SUMMARY OF OBJECTIVES

- Safe electrical systems;
- reliability under everyday and emergency circumstances;
- flexibility and ability to expand; and
- efficient conservation of energy.

PLUMBING AND FIRE PROTECTION SYSTEMS

Drainage

Floor drains with flushing rims should be installed when animal waste is to be flushed down the drain. Although the flushing of these drains is normally manual, it is automated in rooms with automatic cage-flushing systems. Floor drains for receiving cage flushing are generally located against a wall behind the cages. Troughs of cages with provision for flushing are connected to the sanitary drainage system by spilling over a flushing-rim floor drain.

Where flushing-rim floor drains are not required, regular floor drains can be employed. Their strainers should be secured with vandal-proof screws to prevent strainers from being removed by untrained personnel. With this precaution, solid materials will not accidently be washed down the drains and clog them.

Floor drains may not be essential in animal rooms, particularly when such species as rats, mice, guinea pigs, or hamsters are kept in them. If drains are used, the drainpipes should be at least 15 cm in diameter. When drains are not in use, they should be capped and sealed to prevent any backflow of sewer gases. Lockable drain covers help to prevent dumping into drains those materials that should be swept up and removed by other means.

Drainage from rooms housing radioactive animals or experiments must be piped in a separate system and disposed of as required by local authorities. Drainage from rooms containing infectious diseases must be piped in a separate system and sterilized to 110°C before merging with the building sanitary drainage system. One way to sterilize this waste is by collecting it in closed duplex tanks and heating it to 110°C by the introduction of 10 psi (115°C) of live steam underwater, with a detention time of at least 10 minutes. The sterilized waste is then run through a duplex shell and tube cooler to reduce its temperature to one acceptable in the sanitary draining system (usually 65.5°C).

Vents from this separate system must have incinerators, in order to heat the exhaust to 315.5°C before discharging it into the atmosphere. To minimize any possible leaks in the system over the years, the vent piping and the piping to the tanks should be stainless steel with welded joints. If vacuum air outlets are required, they must be connected to a separate system in which incinerators can heat the exhaust to 315.5°C before emitting it to the atmosphere. All water supplies must be protected by an air break, backflow preventer, or vacuum breaker, as required by local code.

Water Supply

Water-hose bibbs should be standard in all animal rooms. Where cages and pens have automatic watering devices, the water piping in each room should be provided with a control station consisting of a pressure-reducing valve, pressure gauge, filter, and control valve. This equipment will make it possible to serve clean water at the proper pressure to the dispensing devices. Water piping to this system must have a backflow preventer or vacuum breaker to prevent possible contamination of the building's domestic water supply. However, under some codes, backflow preventer or vacuum breaker protection is unacceptable; instead, a complete air break in the system is required, as with flushing-rim floor drains and cage-flushing systems.

Large animal cages and pens may need nozzles for flushing pans on the bottom of the unit. A flushing system is often automated by an adjustable automatic timer in the room, which also simultaneously activates the flushing-rim floor drain receiving the water. Some codes require a separate water system for flushing-rim floor drains and cage-flushing devices, with an air break between it and the building's domestic water supply to prevent contamination of the latter. If enough pressure head can be obtained, the separate water system can be a gravity break tank higher up in the building. If a gravity tank cannot be installed, a break tank and booster pump are required.

When sinks in animal rooms are intended for the bathing of animals and are provided with hose sprays, the water supply to these hose sprays must have a thermostatically controlled mixing value to protect the animals from the possibility of being scalded should the pressure in the water piping change suddenly.

Protection Against Fire and Vandalism

All storage areas for bedding and food and all animal rooms in which a quantity of bedding will be placed must have automatic sprinklers installed. In rooms reserved for the housing of primates, all exposed plumbing must be made as vandal-proof as possible.

SUMMARY OF OBJECTIVES

- Safe water supply without possiblity of cross-contamination;
- safe drainage system;
- cleanliness;
- reliability; and
- flexibility and ability to expand.

HEATING, VENTILATING, AND AIR-CONDITIONING SYSTEMS

Environmental Conditions

Individual temperature and humidity control is required for each animal room; heating, ventilating, and air-conditioning systems should ideally be capable of maintaining 18.33-35°C dry-bulb and 50 ± 20 percent relative humidity throughout the year unless specific deviations are required. For surgical areas, relative humidity should be increased to 55-60 percent to prevent static charge accumulation.

Types of Systems

Air-conditioning systems for the animal facility should be independent of any other air-conditioning systems in the building. They may be of the single duct, low- or high-velocity types with terminal reheating, or a double duct, low- or high-velocity model with terminal mixing boxes. These systems can be energy-intensive, but it is essential that a constant volume of air be supplied, and therefore, such energy-conserving systems as those depending on variable volume or air are unsuitable. Where they are economical, heat-recovery devices should be employed.

All heating should be accomplished by air. The use of direct radiation of any kind is to be discouraged, because it present difficulties in housekeeping. If the facility requires supplemental heating in perimeter areas, a radiant-type ceiling is preferred, some standby or backup system is recommended (a bypass of the main house system is probably best).

Odor Control and Pressurization

All air should be 100 percent outside air and it should be sanitized 100 percent for odor control and minimization of pollution within the conditioned space. Although using charcoal filters would permit air to be recirculated, the costs of maintaining and operating them far outweigh the energy conserved.

Animal rooms should have negative air pressure in clean corridors and pathogen-free animal areas, and surgical areas should have positve air pressure in corridors to minimize the possibility of cross-contamination. Infectious areas should be at negative pressure and have an air lock.

The air supply should have a good central filtration, and it should be possible to install high-efficiency final filters at outlets. Air exhaust from infectious areas must be passed through high-efficiency filters and/or incinerated. All supply and exhaust duct-work for these special areas should be welded airtight. The exhaust air is gas-incinerated to 315.5°C for 3 seconds prior to discharge. Exhaust grilles should be low, and they should have hair screens or easily cleanable duct risers.

For air quantities, the guidelines set forth by the American Society of Heating, Refrigerating and Air-Conditioning Engineers have proven satisfactory. However, the use of 100 percent outside air at the present energy costs requires a hard look at each installation; systems employing 100 percent outside air consume tremendous amounts of energy.

Safety Features

- Remote room-temperature monitors;
- alarm systems for air-handling equipment and air conditioners;
- emergency power supply for all air-handling and incinerator equipment; and
- 24-hour monitoring system for safety and alarm equipment.

SUMMARY OF OBJECTIVES

- Constant temperature and humidity that can be adjusted when necessary;
- flexibility;
- reliability--100 percent with backup; and
- energy conservation--heat recovery and controls for efficiency.

DISPOSAL OF SOLID WASTES

Medical and Biological Wastes

Medical and biological wastes are human or animal organs and body parts, carcasses and similar solid organic wastes, dressings, and syringes from hospitals, laboratories, animal pounds, and slaughterhouses. They have as much as 85 percent water and a comparatively low heating value. They are sometimes called Type 4 wastes.

Most commonly encountered mixtures of wastes have been classified and their average moisture and heat of combustion values ascertained. Although several classification systems have been established, the one most useful to engineers working with on-site incineration systems is set forth in the standards published by the Incinerator Institute of America.

Wastes range from Type 0 to Type 6. In the Type 0-4 range, the lower numbers have higher joule values as fired. Types 5 and 6 are industrial wastes and must be analyzed individually.

Methods of Disposing of Medical and Biological Wastes

In general, only two methods have been legally approved for disposing of medical and biological wastes. These are off-site incineration and on-site incineration. Off-site incineration involves transporting the waste to an incineration facility operated by a waste disposal contractor or a municipality. In most areas of the country, however, such off-site facilities are not available, which makes on-site incineration the sole disposal method of choice.

Multiple-chamber incinerators for medical and biological wastes have two or more combustion chambers sized and designed for optimal combustion and minimal emission of air pollutants. Wastes are combusted in a primary chamber, or

furnace. The combustible gases and particles leaving the primary chamber are fully oxidized in secondary chambers by the addition of heat from auxiliary burners. There are two basic types of multiple-chamber incinerators: retort and in-line. The retort-type of multiple-chamber incinerator is a compact, cubical unit best suited for capacities of less than 337.5 kg of waste per hour. Incinerators for medical and biological wastes, which are invariably retort-type units with solid refractory hearths in place of grates, require special design considerations. General-type wastes should *not* be burned in these incinerators; conversely, medical and biological wastes should not be burned in a general system.

Size of Incinerators

The proper size of an incinerator is determined by type, volume, and weight of the waste and length of time allotted for operation of the incinerator. The size of an incinerator for medical and biological wastes is also dependent on the size of the largest animal or material to be incinerated. For example, whenever the largest animal to be incinerated will exceed one-third of the hourly capacity of the incinerator, it is recommended that the burning area (hearth) be increased, in some cases by as much as 80 percent.

When projecting the total weight of the solid waste that will be handled by an incinerator, allowances must be made for changes or increases that will occur over the life of the installation. Many tables list waste-load factors, but they should be followed with discretion. It is often desirable, or even necessary, to make a survey of actual waste loads in an existing installation as similar as possible to the one contemplated.

Other considerations are:

- The daily average weight and volume of wastes to be incinerated. Allow for peak loading, reasonable growth, and unexpected contingencies.
- The hours of operation and burning cycle. Be aware of labor factors and legal restrictions.
- The location of the unit. Consider convenience, stack and flue locations, and clearance.
- Local air pollution codes and methods for meeting their requirements.

Auxiliary Heat

Burners are required for medical and biological wastes. The function of a burner is, of course, to provide auxiliary heat when and if needed. The higher the moisture content of the refuse, the greater the auxiliary heat requirements. The recommended auxiliary fuel is natural or liquified petroleum (LP) gas (butane or propane). However, if gas is not available, No. 2 fuel oil may be used. Heavier grades of oil should not be used because they may cause operating difficulties.

A substantial input of natural or LP gas (butane or propane) is required for combustion of medical and biological wastes because of the high percentage of water. The gas combustion should supply 11.7-16.4 million joules/kg of waste burned to the primary chamber and 7.0 million joules/kg to the secondary chamber. This heat can best be supplied with burners for premixed gas, which are incorporated to provide the combustion air. The required burner capacity in joules per hour is the stated value times the incinerator capacity in kilograms per hour.

AIR POLLUTION

Incinerators for medical and biological wastes can produce highly objectionable emissions of fly ash, smoke, gases, and odors. Emissions of fly ash tend to be inconsequential in this type of incinerator, but odor emissions may be very great. Visible smoke from the retort incinerator is highly repugnant on aesthetic grounds, and it is especially undesirable from crematory furnaces. Poorly designed incinerators, with inadequate mixing, temperatures, and residence times, discharge highly objectionable contaminants into the air.

Complete combustion is most readily achieved by the employment of two-stage combustion. In a well-designed incinerator based on this principle, the waste is burned in a primary chamber. The products of combustion are then mixed with additional air and passed through a secondary chamber for combustion of any unburned fractions that may have been drawn into the flue gas from the primary chamber. Although two-stage combustion is applicable to all types of waste, its use is especially important when incinerating medical and biological wastes because of their large water content. A well-designed incinerator for these wastes is a multichamber unit having primary and secondary combustion chambers with a mixing chamber installed between the two.

Flue-Gas Scrubbers

Although it may be an integral part of the incinerator, the flue-gas washer (scrubber) is generally a separate piece of equipment located between incinerator and chimney. Products of combustion pass through a series of spray patterns of water created by stragegically located nozzles. Because of the increased resistance caused by the water, a fan to induce drafts is usually required at the outlet side of the washer. Local clean-air codes must be consulted inasmuch as one municipality may require a washer on all incinerators, another may require them on all units with a capacity of more than 180 kg/hour, and another may mandate that washers be employed when incinerating only unusual types of waste.

OPERATING AND MAINTENANCE

For the laboratory animal housing facility to serve its desired purpose, it must be continuously and properly served in a safe and reliable manner by the various mechanical and electrical systems

described. Accordingly, proper operation of the systems and a proper preventive maintenance program must be instituted from the opening day of the facility.

The first step in such a program is to provide all operators with a complete operating manual for the various systems. The manual should have diagrammatic layouts of the systems, indicate proper control settings and instrument readings, and contain a check list of likely trouble spots in the event that the systems are not performing correctly. The manual should also list the specific locations of all important operating and safety elements, indicate the origin of all electrical circuits and areas which they serve, and present the operators with a total view of their facility. The manual is useful not only at start-up and for general day-by-day operation, but it becomes an important tool in training new personnel.

A second important ingredient in proper operation is the preventive maintenance manual. This manual, in addition to containing a recommended inventory of spare parts, should explain the frequency of maintenance necessary for all major equipment. It should also describe daily routine inspection tours and list the items of equipment to be inspected and logged in during those tours. No operating and preventive maintenance program can guarantee 100 percent reliability, but the use of operating and preventive maintenance manuals will go a good way toward achieving that goal.

CONCLUSIONS

Proper design, installation, operation, and maintenance of the mechanical and electrical systems serving the laboratory animal housing facility are essential to achieving an efficiently functioning facility. The key objectives are reliability, flexibility, simplicity, and economy. Although no substitute can be found for the advice of experienced professional designers and engineers, it is hoped that this paper has provided a checklist of systems and detailing that will prove useful in any future planning endeavors.

Integrating Psychosocial Objectives into Design

DONALD J. CONWAY

In this paper, I am going to talk about animal laboratories as work environments and what makes them better places for people. To start with I am going to ask you to close your eyes for a moment and picture the "environment." What did you see? Forests, sunsets, and flowers? Or did you imagine cities, playgrounds, and high-rise apartments? If you pictured the buildings or other man-made objects, you are a bit unusual. Most people think of the "environment" as the *natural* environment. Yet we and people who work in animal laboratories spend something like 95 percent of our time in an environment somebody consciously designed and built.

What makes an environment a better place for people? Very simply, a better environment--a better place for people--is a place that meets people's *needs*. What do you and I need from the places where we live and work? Or, in the context of this conference, what do people who spend so much of their time as employees in animal laboratories need from those places? Most of us don't really know. We know that we feel good in some places, but no so good in others. But we all have environmental needs, and an increasing number of social scientists are identifying them for us.

The most basic environmental need we all share is the need for shelter. Our environments must provide protection from the elements and other dangers. Of this particular need you are well aware. When we focus our attention on animal laboratories, the problems of disease control and bacterial infection seem to be well recognized by this audience. Beyond shelter and its health-related aspects, however, work environments must be places in which workers can conduct their daily activities, many of them more than simply performing occupational tasks in a safe atmosphere. I am referring to the psychosocial aspects of the work setting, and the balance of my remarks will be directed to this aspect of animal laboratory design.

Once our requirements for shelter are met, we discover other needs. After shelter, our first psychosocial environmental need is for territory. We need to stake out and mark a piece of space of our own and, somehow, show that it is exclusively ours. Sometimes we exhibit territorial behavior. For instance, a European concierge lets us know by sitting in her courtyard or entryway that this building and its spaces belong to her. We use objects as well as behavior to stake out our territory. Thus, when we edge our yard with a row of stones or trim the shrubbery, we are using both objects and behavior to define a territory. Examples of animal laboratory workers who employ behavior and/or objects in this respect may come to mind. The need to establish and mark a territory is very strong. If laboratory workers are denied that opportunity by some failure in the design or through some policy of management, they will enter into a sort of guerrilla warfare to express this need.

We all share the need for privacy, which doesn't mean being alone all the time. Rather, it means being able to control how much we are alone and when we are alone. We need to control the flow of people through our lives. We need to be able to say, "You can't come in here now" or "I control when you can enter into my space." As we do with territory, we control and maintain

privacy through behavior and with objects. Thus with body language, such as bending over a piece of work or turning our backs to a door, we say, "Don't disturb me." The objects we manipulate to control social interaction are everywhere around us in the physical environment. When you or I go into our offices and close the door we have used a part of our physical environment to regulate social interaction. Even under the poorest conditions, people will find ways and objects to say, "Stay out." Correspondingly, if workers in laboratories are denied privacy through omissions in design or management, they nevertheless will find ways to achieve it.

Another environmental need we all share is the necessity for personalization. Personalization is our way of insisting on our own identities in the world. We personalize in our styles of grooming and dress, and we personalize by the use of objects. The photographs, diplomas, paintings, or whatever that we all install in our offices are good examples. In the same way, when teenagers hang posters in their rooms they are personalizing. Most of us individualize our belongings and our spaces, whether they be the humblest of shacks or the most elegant of houses. Thus we indicate to the world that an *individual* lives here. Sometimes, however, employees are not provided with a physical design compatible with personalizing their work spaces or management does not give them the right to personalize. Then, as they do to achieve territory and privacy, they will take the means at hand to personalize as best they can. Undoubtedly, the teenagers writing their names all over the New York subway cars are saying, "This is ME." Interestingly, the graffiti on the New York subway cars also expresses a sense of territory, since the names are almost always accompanied by a number. The number indicates the street or city locations where these youngsters live and hang out.

We also have the need to socialize. We can best observe this need by looking at it as three levels. First, we need to have intimate one-to-one exchanges with close acquaintances. Second, we need to socialize in small groups, i.e., in groups of 10 or less. Finally, we need to socialize in groups comprised of more than 10 people. Our environments, including the places where we or animal technicians work, must accommodate these different levels of socializing. They must also allow us the space to engage in some vicarious socializing--the behavior we all know as "people watching." Sometimes humans need to sit apart and observe what goes on in the world before them. This watching is an educational process through which we learn appropriate social behaviors for various environmental and social situations. Again, animal laboratories as work environments must accommodate all these forms of socializing behaviors or employees will take matters into their own hands.

The fifth need we all share is the desire for mobility. In its simplest context, mobility allows us to get from point A to point B. Many parts of our designed environment--streets, corridors, sidewalks, bus stops--all make this possible. We also need mobility in order to receive a *variety* of environmental experiences, to be able to change the character of our environments. We must be able to control the amount of stimulation we experience--to be able to go from some place that is too noisy, too crowded, or too hot to a quiet or less stimulating place and back again. The literature on confinement and social isolation is quite rich, and it is easy to imagine the "pent-up" feeling of employees in animal laboratories if they do not have adequate opportunities to change their environmental stimuli fairly frequently. The design of the laboratory and the management policies that control how it is used must allow for variety.

The next environmental need that must be considered is that of communication. Workers obviously need to be able to communicate with each other to do their jobs, but we also require communications from the physical environment to help us go about our daily affairs. The physical environment carries a great deal of information to us through combinations of words, pictures, and symbols. Such communications tell us how to behave--they give us cues about what we should do in the environment. For instance, the simple presence of a wastebasket easily conveys the message, "Throw trash here." More familiar messages reside in the white lines on the road and at intersections: "Cross here" is said to pedestrians and drivers are exhorted, "Watch for pedestrians." Through symbols, objects, and words, environments tell us to stop, go, eat, sit, and so on. In animal laboratories, for example, signs and symbols tell us of radiation hazards and contaminated areas. This need for the physical environment to carry messages to us creates difficulties for architects and others concerned with building an environment because we must make sure the messages we send are clear and understandable. Sometimes we get too much information from the environment; for example, a very busy road intersection with its traffic signals, bill boards, and store signs. Sometimes the information is unclear and sometimes it's confusing. At times we don't get enough information, such as where to locate a fire hose, a lack that could turn into a crucial liability in an emergency.

Thus we have seven common environmental needs--the basic requirement for shelter, followed by territory, privacy, personalization, socializing, mobility, and communication. Unless our environments contribute to the expression of these needs, we're often unhappy or tense or anxious, although we may not know just why. As an architect, I am in the business of helping people to construct environments in harmony with their psychosocial needs. Of course, many others help: engineers, landscape architects, zoning board officials, managers, and janitors all make decisions about the man-made environment. Our decisions can frustrate or help satisfy the requirements of the individual. With regard to these general environmental needs that we all share, one may well ask, "What do psychosocial factors really mean in the animal laboratory

and what can I do about them?" If adequate provision for these human needs has not been made, employee effectiveness and possibly morale will not be optimal. Competitive salaries, fringe benefits, and pleasant colleagues may nullify some environmental deficiencies, but they will not overcome a totally unresponsive environment. For example, the U.S. Army came to realize the poverty of the troops' environment when the draft ceased and it was faced with the necessity of maintaining an all-volunteer army. The Army sees a direct relationship between the design of its facilities and its employee turnover rates and re-enlistments.

Think of the investment your organization has made in training and educating its employees. Every time an employee leaves and another one has to be trained and absorbed into your organization, the total personnel cost of your operation goes up. To the extent that a relationship exists between deficiencies in animal laboratory design, employee satisfaction, and employee turnover rates, responsive environmental design is an area worthy of management's attention. Equally as important from management's point of view is the physical setting as a help or hindrance to the achievement of the institution's mission. Without consideration or concern for the kind of issues I have presented and without adequate accommodation of these human psychosocial needs, the organization's "home" will be less than optimal and possibly counterproductive to the achievement of its goals.

Obviously, none of what I have said so far dilutes the necessity for a building to meet its normal functional and task-related requirements. The psychosocial needs of animal laboratory workers should be thought of as being in addition to task-related functions. Adequate provision for these needs will make the qualitative difference between a building that is merely habitable and one that is a very comfortable and satisfying place in which to work.

What can you do about all this? In your own work setting, you can insist upon your own "environmental rights," so to speak, of territory, privacy, personalization, social spaces, mobility, and adequate and clear communcations. Second, to the extent that you are involved in remodeling, maintenance, or the design of new facilities, you can ride herd on your architect and see to it that he or she pays close attention to psychosocial factors on behalf of the employees and the organization that expends so much money for an environment's design, construction, maintenance, and management. Third, you can see to it that adequate and thorough programming is carried out for any new facility with which you are involved. That is, plans must be developed for both a task-related, functional program *and* a behavioral program that shows the organizational goals and the employee social structure that must be supported and which considers at least the needs I have pointed out. Fourth, and this imperative applies mainly to medium and large institutions--ones that will be building, owning, and maintaining their own structures--you can insist upon a post-design or post-occupancy evaluation of your facility to assess how well it is responding to the needs of its users. Think of this activity as an analog to the periodic checkup or physical examination you get to see how well your own body is working to help or hinder you from living well. This comparison is a valid one because our physical environments affect us biologically, psychologically, and socially as we do our work. If our environments meet *all* of our needs, we are happier, more productive, less anxious; and that is a "better environment," an environment for people.

Discussion

MORELAND: I have a question for Mr. Zigas. I believe you stated that the incinerators should only be used for one type of waste. Isn't it possible to provide incinerators that will burn both Type 1 and Type 4 or other combinations of wastes?

ZIGAS: Yes, but it is going to be more expensive. What we have been doing (we are making a study of this possibility for the Mayo Clinic) is burning all our wastes. Of course, we are working on a good size heat-recovery situation. It is somewhat expensive but it probably becomes cost-effective because of the heat recovery. Most hospitals are now separating their medical and biological wastes from other wastes. All it requires is good housekeeping. The rest of the so-called garbage or dry waste is compacted and shipped away.

POVAR: I have a question about relative humidity in animal rooms. You said that a 50 percent relative humidity was optimal. It was explained to me that a relative humidity control of 50 percent plus or minus 2-5 percent would cost as much as our entire facility and then some. Would you care to comment on that?

ZIGAS: I think what we are talking about is a range of humidities. I do not think you would want to have two adjacent rooms with widely differing relative humidities. I think if you allowed a 5 percent swing, 45-55 percent, by using a reheat system, that you would attain very close control on the relative humidity. Extremes of humidity would be handled on a room-to-room basis. If you need a high humidity, you would install humidifiers and spray clean steam into the room. If you want an extremely dry room, then you would probably want to have a recooling as well as a reheating system. It is not that expensive, because by having individual room controls with a constant air volume you are limited to a terminal reheat or a dual-duct, terminal mixing-box.

HICKEY: I am Dr. Hickey with Mead-Johnson. I also have a question for Mr. Zigas. I have heard a rumor that recessed lighting has fallen into disfavor with the Occupational Safety and Health Administration because of problems with fire hazards. Would you please elaborate on this?

ZIGAS: The only reason our first preference in lighting is not recessed lighting is that the staff or the operators or the owners of a facility generally do not like to have a hung ceiling because it might house vermin. The few facilities I have been closely involved with have exposed ceilings, and the lighting fixtures are waterproof and completely enclosed so that the room can be hosed down.

KENTNER: I am Donald Kentner from Schering Plough. I have questions for Dr. Woods and Mr. Zigas. To Dr. Woods, you mention three methods of preventing cross-contamination: air dilution, cage separation, and filter barriers. Would you include unidirectional mass air movement as a fourth means of separating cages?

WOODS: Yes, if you are moving high volumes of highly filtered air, it would be the same as putting a filter cap on a cage. That would be very acceptable--in fact, it is a common practice.

KENTNER: Mr. Zigas, you stated that heat sterilized pathological water waste must be cooled to 62°C before dumping into sanitary sewers. Should we also be concerned with superheated

water (82-115°C) dumped from cage washers?

ZIGAS: I was afraid that this was going to come up. Most municipal codes require that the wastes be no higher than 62°C, and what you say is correct. We generally do not cool the wastes from the cage washers. However, I do not think you will find a waste that is much higher than 62°C. I am not quite sure of the exact temperature, but certainly it is not as high as what you would get from a sterilizer, which would certainly be at the boiling temperature when it was released. In a cage washer, the temperature of the water should be about 82°C so, by the time it is dumped, the water is pretty close to 62-71°C. You must also remember that this hot water is being drained into some kind of pipe system. If the joints in the system are old, caulked, lead joints, the high temperatures will cause leaks.

JONAS: Most of us have to deal with air circulation or air distribution within the rooms. You alluded to the problem with anesthetic gases and coupled that with a statement that you prefer a 100 percent exhaust system. So, I have two questions. One, are you implying that there has been evidence that emissions of hazardous gases have only occurred in operating rooms that have been on recirculation and not in areas serviced by 100 percent exhaust systems? The second question relates to my experience that the so-called 100 percent systems have very poor distributions within animal rooms and high turbulent flows; thus we end up with tremendously high particulate counts. The question is, is there any other method in use which under certain circumstances may have advantages and yet employs recirculation? In other words, do you have a hard and fast opinion on 100 percent exhaust systems eliminating all other systems or do you think there is still some room for technological development?

ZIGAS: The first part of the question concerns the operating room and anesthetic gases. I do not know if a relationship exists between recirculated and 100 percent outside air, but I do know, and there is enough evidence, that you would be wise not to recirculate air because recirculation merely increases the volume of pollutant. In an operating room, I would certainly want to go to 100 percent outside air. I just do not think that the economy of the energy conservation is worth it.

As for the distribution of air in an animal room, it is a real mixed bag. If there is no hung ceiling, there are two choices. Sidewall registers can be fed from the corridor or exposed ducts with overhead diffusers can be used. Laminar flow is more expensive. Now, how much air do you put into the room? Technically, the amount of air that can go into any room is really determined by the heat load or the gain in the room. Theoretically, that is all you should need. Yet one mustn't forget the practical consideration of how you get the distribution in the room. If, for instance, the animals in the room give off very little heat and illumination is low, the amount of air required to pick up the heat for air conditioning would also be very low. However, there is a point that you do not want to dip below, because then it becomes difficult to maintain circulation. However, the rule of thumb of 15 air changes an hour should be reexamined, because at 100 percent outside air, it means a huge number of joules.

WOODS: I would like to add one comment to Mr. Zigas' answer. In the paper I presented, the airflow into the room is normally called air supply. That air exchange does not speak to the quality of the air, whether it be outside or recirculated air. Now, the 100 percent outside air, as I understand the history behind picking the 15 air changes an hour, is a rule of thumb; that number has worked in the past and therefore we tend to retain that number, because we do not have anything else to work with. Yet when we use 100 percent outside air and 15 air changes an hour, in addition to the energy expenditure, the quality of the outside air may be worse than the recirculating air.

Under those conditions, it is necessary to pay attention to ventilation standards that require filtration of the outside air before it can be considered ventilated. You must deal with the quality of the air, that is, you must regulate both the particulate and the gaseous concentrations of the air that you are using for ventilation.

As far as the thermal aspects are concerned, helium can be used for thermal exchange. We could get the heat exchange by using any kind of a medium. We need air-quality control to maintain the gaseous and particulate concentrations within acceptable limits. So, we talk about air supply into the room and about the quality of the air that is coming from outside or that is being recirculated. This air has to be treated before it becomes acceptable to supply the space.

Now, as far as the diffusion within the space, I think the interpretation was correct. We have enough evidence to design systems based on high sidewall or ceiling diffusers and each should be able to provide uniform flow for minimizing stagnation and stratification in laboratory animal facilities if the Air Diffusion Performance Index (ADPI) system of design is used in selecting the size of the diffusers and their location.

III

Containment of Hazardous Agents

Introduction

DONALD G. FOX

The focus of this third session of the symposium is on the unique biohazards encountered in the context of laboratory animal housing. The papers in this session are, in large part, a continuation of other pioneering work in the area of environmental control. By design, the area of radioisotopes was omitted; the subject is enough in and of itself to warrant another entire session. Persons interested in earlier work on the health and safety aspects of the research laboratory and the technology of decontamination, incineration, and sterilization are invited to consult the following literature:

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As can be seen from our agenda, the National Cancer Institute (NCI) has a keen interest in this symposium. The topics under discussion are those issues our grantee design teams are facing daily. We hope that this symposium will enable you to understand better our criteria and for us to understand better the difficulties you have found in maintaining laboratory animals. This symposium is only one of several programs supported through the concern of the NCI for laboratory safety. Training programs for the safe handling of oncongenic viruses, recombinant DNA molecules, and chemical carcinogens are now being sponsored regularly and without cost to any laboratory worker or safety officer who wishes to attend. The Institute has also prepared a number of film strips and manuals on laboratory safety. Is is hoped that this symposium will lead us toward some answers. I am pleased to participate in these activities and believe that this program will be an important step in resolving our shared concerns.

The Need for Hazard Containment

DONNA VREDEVOE

CONSIDERATIONS THE RESEARCHER MUST FACE

Occupational health and safety hazards are rapidly becoming a national crisis, and there has emerged a new awareness of the increasing numbers and types of hazards in the research laboratory. Fortunately, some researchers are anticipating potential dangers and are participating in the creation of guidelines for handling potentially harmful agents (Berg *et al.*, 1974, 1975; National Institutes of Health, 1976). Previously a researcher beginning a new project would focus on ways of receiving funds for personnel, equipment, animals, etc., and acquiring sufficient space for the endeavor. Now the researcher must consider whether the project is safe for staff, subjects, and neighbors in the laboratory environment. Space must be adequate not only in terms of size, but also must be equipped with means for containing hazardous material and protecting personnel.

Today, researchers designing biocontainment facilities must decide whether facilities should be of Level P1, P2, P3, or P4 (National Institutes of Health, 1976); whether safety hoods should be of Class I, II, or III, and then of Type 1 or 2 (National Institutes of Health, 1974); whether the etiological agent under study should be handled as a low, moderate, or high risk (National Cancer Institute, 1974); and whether it is of Class 1, 2, 3, or 4 (U.S. Public Health Service, 1974; Stark, 1975); whether solid and liquid wastes should be disinfected, incinerated, decayed, decomposed, or diluted; and whether exhaust air should be filtered, incinerated, ultraviolet-irradiated, or simply discharged (Runkle and Phillips, 1969; National Institutes of Health, 1974). In asking for guidelines to help make these myriad decisions, the investigator is often confronted with the fact that in many cases, no one knows for certain which decision is best and that he or she can only make informed judgments. For example, sometimes different terminologies are used for different etiological agents. Fortunately, attempts are being made to bring some order to this situation, and subsequent papers will speak to these terminologies and decisions. This paper is intended as an overview of the legal and moral responsibilities of the investigator, the research personnel, and the institution in regard to hazard containment. Some ideas will be introduced as to how responsibilities might be assumed and delegated. Solutions to problems cannot be offered yet; only directives for achieving these solutions can be presented.

Once investigators initiate research, they and their staff must evaluate for safety all aspects of the research project. Hazards difficult to detect must be sorted out. In animal research, "normal" animals must be viewed as potential carriers of agents that may be infectious for humans or other animal species (Baum *et al.*, 1966; Boulger, 1966; Friedmann *et al.*, 1971; Hull, 1973; Lennette, 1973; Rowe, 1973; Whitney, 1975). Solvents, airflow systems, and water sources may harbor unanticipated hazardous biological agents or chemicals. Although environmental hazards of ionizing radiation are generally recognized, risks from other types of materials and equipment are not as well known. For example, researchers may not be aware that laser beams that cannot be felt can nonetheless damage the

retina of the eye. Indeed, even the blinking reflex cannot respond before damage has been inflicted ("Laser Eye Safety," 1974). Standards for protection of people against nonionizing radiation, including ultraviolet, infrared, visible light, microwaves, radio-frequency waves, and lasers are being developed (Ham *et al.*, 1970; Swope, 1970; Vassiliadis *et al.*, 1970; Michaelson, 1972, 1974; Ryer, 1975; Youmans and Ho, 1975).

Perhaps the most complex assessment to make is the evaluation of the hazards of working with potentially oncogenic agents. With chemical carcinogens, the hazard potential is better established (Miller, 1970) than with potentially oncogenic viruses. For viruses, the hazard is often inferred from animal studies or rare human situations in which viral etiology of cancer is suspected (McGrath *et al.*, 1974; Gallagher and Gallo, 1975; Klein, 1975; Holland, 1976; Kessler, 1976; Stutman and Herberman, 1976; Vianna, 1976).

Thus, investigators are confronted with a plethora of potential hazards as they contemplate initiation of new research. Awareness of hazards has increased among scientists, technicians, and the public because the news media as well as scientific journals now report dangers ranging from damage to the fetus of pregnant laboratory workers to death and disease years after exposure to substances in laboratory environments (Cimons, 1976; Cooper and Steiger, 1976). In 1971, the Occupational Safety and Health Act (Public Law 91-596) became effective. The Act pertains to every employer in the nation engaged in business affecting interstate commerce. Although the regulations originally were developed by the Department of Labor for application to industry, they are now being extended to the research laboratory. Costs of making environments safe for work with potentially hazardous agents can be high. Federal granting agencies are beginning to recognize this need, but the costs of safety equipment and construction of specially equipped laboratories often exceeds the available funds for research. In summary, researchers are faced with defining hazards and containment procedures, meeting existing laws and recommendations, and funding the new safety equipment, laboratories, and procedures.

REGULATIONS AND RECOMMENDATIONS

Before 1970, congressional action related to occupational safety and health was directed at specified industries--e.g., the Federal Metal and Nonmetallic Mine Safety Act, Federal Coal Mine Health and Safety Act, and Contract Workers and Safety Standards Act. Even collectively, all the federal safety legislation passed before 1970 was not applicable to most workers. During the 1960's, organized labor and other individuals recognized the need for stronger, more encompassing federal legislation to cover occupational safety and health. A new national policy was established on December 29, 1970, when the Occupational Health and Safety Act of 1970 was signed. The Act, sometimes termed the Williams-Steiger Act after the coauthors, Senator Harrison A. Williams (D-New Jersey) and Congressman William Steiger (R-Wisconsin), became effective on April 28, 1971. The federal program is coordinated by the Department of Labor. Both the Occupational Health and Safety Act and the Occupational Health and Safety Administration are referred to as OSHA in scientific publications (Robinson, 1972; U.S. Department of Labor, 1975). Executive Order 11612 of July 26, 1971, and then Executive Order 11807 of September 28, 1974, indicated the special obligation of the federal government to set an example for all employers by providing safe and healthful working environments for its employees.

The states have been encouraged to initiate their own occupational health and safety plans. However, such plans must be submitted to OSHA for approval and must be at least as stringent as the federal standards. If a state plan does not cover all issues covered by the federal program, then it must surrender such issues to federal OSHA.

OSHA requires that employers furnish to employees a place of employment free of recognized hazards that are causing or can cause death or serious physical harm. Specifications for safety range from fire suppression equipment to fixed ladders to chemical carcinogens (Occupational Health and Safety Act, 1970; "Congress Grapples with Chemical Safety," 1974; "OSHA's Controversial Carcinogen Standards--How They Evolved," 1974; "Regulations on Chemical Carcinogens," 1974; U.S. Department of Labor, 1975). Perhaps, of most significance to laboratory research, is the impact that OSHA has had on federal agencies and research and educational institutions. One of the best working models for developing safety practices and environments has been that of the National Cancer Institute (NCI) in its work with chemical carcinogens and oncogenic viruses. NCI has developed recommendations for handling such agents in consultation with laboratory workers, safety officers, and administrators throughout the United States (National Cancer Institute, 1974, 1975).

Equally as compelling as the legal responsibility for safety is the moral responsibility of investigators and institutions to furnish safe working environments for employees. In addition, responsibility extends to concern for neighbors within the institution and community. The obligation includes informing employees as to the potential risks involved in the research and ways to minimize those risks. At present, we have no legal requirement concerning the manner of providing such information to employees, but it is likely that there will be a move to supply such information in writing with a verbal explanation and written documentation by employer to employee. One could look to the types of written statements now requested of human research subjects as models for informed consent by employees. Moral obligations extend not only to minimizing anticipated risks, but also to administering emergency treatment to those who

have experienced injury. In cases involving contamination of articles that can be removed from the work area, responsibility extends to eliminating risk of removal of such materials.

DELEGATION OF THE RESPONSIBILITY FOR SAFETY

OSHA defined the responsibility of the head of each federal agency to establish and maintain an effective and comprehensive occupational safety and health program as defined by that Act. Specifically, the head of each agency was required to provide safe conditions of employment and safety equipment and maintain records of occupationally attributable accidents and illnesses (Section 19). Executive Order 11807 strengthened the occupational safety and health programs of all federal agencies and established the Federal Advisory Council on Occupational Safety and Health to assist the Secretary of Labor. Title 29 of the *Code of Federal Regulations*, Part 1960, "Safety and Health Provisions for Federal Employees" (1974) specified requirements for safety programs. Heads of agencies were instructed to establish safety and health committees for the purpose of advising agency officials of their responsibilities under the agency's occupational safety and health program. The National Institutes of Health have specified responsibility of management, principal investigators/supervisors, and individuals in biohazards programs (U.S. Public Health Service, 1974).

Some suggestions are offered by this author for further definition of these responsibilities. In educational institutions, safety committees for the interpretation of OSHA regulations should be composed of principal investigators representing a variety of research activities, health and safety officials, and technical and administrative personnel. Because safety cannot be defined exclusive of establishment of risk, these committees could have the following activities:

- working with principal investigators to determine potential risks in research;
- approving safety procedures developed by the principal investigators and appropriate consultants;
- delegating responsibility for monitoring the safety of research projects; and
- maintaining records of review of safety procedures for research projects.

Executive Order 11807 mandates that heads of agencies designate a person to be responsible for the management and administration of the agency's occupational safety and health program. This person becomes a key official in the administation of safety programs as he or she works with the agency head to establish and implement policy and set goals, procedures, and priorities for occupational safety and health programs within the agency. The principal investigator may be in the best position to:

- assess potential risks of agents involved in the research program;
- determine, with consultation if necessary, factors in susceptibility of personnel to exposure to agents;
- determine, with consultation if necessary, short-term and long-term consequences of exposure to agents;
- develop, in cooperation with the institution, safe procedures for use of the agent;
- put into effect institutional safety policies for his or her research program in regard to agents already recognized as hazardous;
- acknowledge potential hazards and initiate corrective action;
- be a role model for safety practices;
- cooperate with safety officers in investigation of accidents and reporting and in initiating corrective action and development of revised safety procedures or installation of safety equipment;
- develop techniques for disposal of hazardous materials;
- create a plan for safety within his or her research group that includes delegation of responsibility for maintaining safety equipment, checking safety procedures, and proposing recommendations for revision and updating of laboratory safety; and
- request from management safety equipment not provided.

The laboratory workers working with the principal investigator are responsible for complying with safety rules and procedures relating to their work. Responsibility extends to reporting all accidents or unsafe conditions. Perhaps the most important obligation of workers is to maintain peer support for a positive attitude toward safety.

THE HUMAN ELEMENT IN HAZARD CONTAINMENT

All individuals involved in work with hazardous substances should recognize conditions that could predispose to unsafe conditions. Certain variables relate to the physiological state of the laboratory worker. It might be advantageous to screen laboratory workers for preexisting conditions that might result in discomfort or increased risk. For example, individuals with specific allergies obviously should avoid repeated exposure to allergens if they are present in the laboratory environment. If risks can be minimized through desensitization, this technique could be recommended. If such physiological alterations are impossible or unacceptable to the worker, then barrier systems must be used or the worker may choose to be relocated.

It is frequently mentioned in laboratory safety manuals that pregnant women should not work in laboratory areas where certain hazards may exist (Hellman, 1969; U.S. Public Health Service, 1974). Although the rationale for this recommendation is clear, the ramifications to the laboratory worker can be far-reaching. It may be necessary to take a harder look at this and other recommendations that exclude persons with certain biological conditions or

sensitivities in an effort to minimize the effects of curtailment of potentially hazardous work on the career of the person involved. Consider the situation of pregnancy. In the case of a female principal investigator, the consequences of curtailment of certain aspects of a research effort could have long-term effects in terms of research programs, funding, and continuation of positions for support personnel. It is frequently difficult, if not impossible, to delegate an entire aspect of the research effort during pregnancy. Perhaps laboratory safety and procedures should be improved to the point where pregnant women can continue to function in environments in which potential hazards are minimized to such an extent that the risk to the mother and unborn child would be no greater than would exist in another work place or setting. Laboratory safety should not be of such limited design as to provide protection solely to healthy persons without predisposing risk conditions. It should be sufficient to protect individuals in temporary higher-risk situations such as pregnancy. To do less is to create barriers to employment.

The psychological state of the laboratory worker plays a large role in laboratory safety. The initial concern is that the worker perceives a potential hazard as such. A principal investigator cannot assume that all personnel perceive hazards similarly, and he or she should work to identify criteria that are used in defining potential hazards as well as the limits of the definitions. It becomes particularly difficult to instill new attitudes when a laboratory "converts" to a biocontainment laboratory without concomitant changes in experimental etiological agents. For instance, in our recognition of potentially oncogenic agents, we are passing through transition stages in which agents that yesterday may have been used openly in the laboratory must now be contained. The chemical carcinogens are excellent examples. When such a realization occurs, nevertheless, there is a tendency to think that, since no immediate negative consequences resulted when agents were handled openly, there is no reason to contain them now. However, it is the responsibility of the principal investigator and his or her research group to change such attitudes once new or potential hazards are pointed out. New patterns of behavior must be adopted. The development of behavior patterns appropriate for laboratory safety has been outlined (J. Martin, 1976). The whole complex of the psychosocial factors that impinges upon laboratory safety is an intricate and critical subject (Lehmann, 1975).

SUPPORT FOR SAFETY

Establishment and maintenance of safe practices in the research laboratory require administrative, financial, and peer support. Intraresearch group support can function successfully with goals such as fostering of a positive attitude toward safety, positive reinforcement for those following safe procedures, open communication systems for reporting accidents and unsafe conditions, and developing between workers and supervisors a mutual understanding of what could be a hazard.

LEGAL RESPONSIBILITY AND CONSEQUENCES

With literally thousands of safety regulations applicable to many laboratory settings, the institution and investigator have the responsibility to sort out and implement relevant regulations. OSHA regulations for violations include penalties ranging from fines to imprisonment (Robinson, 1972). In research, one of the most direct and immediate sanctions is the loss of funds for research. Funds can be lost or revoked when applications are not approved because safe environments for the research cannot be assured.

The Occupational Safety and Health Administration currently lists some 4,400 safety and health standards. With an ever-growing number of regulations, legal responsibility witin institutions becomes a complex matter of defining pertinent regulations and then establishing compliance. By beginning from the position of providing safety as a prerequisite, or at least a concurrent endeavor, to research, one can move more readily to the positive benefits of safety rather than becoming immersed in legal defense and possible penalties. The positive aspects of safety programs are many. Research can progress more rapidly, even when adhering to more cumbersome biocontainment techniques, if experimental systems, personnel, and the research environment are protected. Wiser use can be made of materials, particularly research animals, because repetitions of experiments are avoided. As employees realize that their safety is a concern of management, respect for supervisors and administrators grows.

THE FUTURE

Many challenges lie ahead in establishing and maintaining safety in the research laboratory. Some recommendations stand out as priorities:

- Better communication should be developed between worker and supervisor in regard to safety. Communication recently has been focused more heavily on interchanges between management and the principal investigators regarding their responsibility.
- Intra- and interinstitution policies must be organized in better ways for monitoring safety, particularly in new areas such as carcinogin control. What are the best methods of disposal for agents? How can inactivation or removal of agents be monitored?
- At the inception of their employment, it may be necessary to obtain informed consent from workers assigned to biohazard areas.
- The present attitude of screening out employees with particular vulnerability to risk situations (e.g., pregnancy, temporary immunological deficiency) should be examined in

terms of how this practice may slow the progress of the career of the people involved. Consideration should be given to an effort to change the research environment to a higher level of safety, rather than to remove the personnel.

- Greater attention should be paid to the psychosocial impact of working in biocontainment areas.
- A more realistic pattern for legal enforcement of regulations must be developed. If more than 4,000 regulations, some outdated by the time they are written, are enforced in more than 4 million establishments, the system can so overwhelm the investigator that the goals of safety and health are lost.
- Scientists may have to assume more responsibility for identifying potential hazards and creating means for containing them.
- Means for funding safety equipment, facilities, regulatory personnel, symposia, and programs must be more readily available.
- The terminology of safety (e.g., P1 to P4, Class I to Class III; etc.) should be clarified and simplified as much as possible. Educational programs in safety--such as that developed by the University of Minnesota in collaboration with the National Cancer Institute--should be encouraged.

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Hazards Associated with Infected Laboratory Animals

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The purpose of this paper is to bring together published information on the biohazards of working with infected laboratory animals, a subject that has been extensively documented (Berry and Kitchen, 1931; Bedson, 1940; Mitchell, 1959; MacPherson, 1960; Kirchheimer *et al.*, 1961; Jemski, 1962; Hill, 1963; Hull, 1963; Phillips and Jemski, 1963; Prier *et al.*, 1964; *Symposium on Infections of Laboratory Animals Potentially Dangerous to Man*, 1964; Darlow, 1967; Fiennes, 1967; Graham-Jones, 1968; Tobin, 1968; Edward, 1969; Hellman, 1969; Hummer, 1969; Perkins and O'Donoghue, 1969; Simmons, 1969, 1975; Kruse and Wedum, 1970; Quist, 1972; Wedum, 1974; Kawamata and Yamanouchi, 1976). Material will be presented in a way that I hope will be clear to concerned persons who may have little or no training in the biological sciences. The discussion focuses upon the use of "low-" and "medium-risk" biological agents because most research activities do not involve "high-risk" agents. Some attention will be paid to defining levels of risk for hazardous agents, and to pointing out the disparities among existing classification systems. Finally, some general principles for controlling and containing biohazards of infected laboratory animals will be reviewed.

THE HAZARDS

The overall risks of working with infected laboratory animals fall into four categories. First, animals may be infected with human pathogens and cause infections to spread among laboratory personnel. Such infections can range from mild or inapparent infections to long-term disabling disease or death. It is significant that, in several instances, the first indications that certain microorganisms are pathogenic for humans have been discovered as a result of infections acquired in the laboratory. Another risk in working with infected laboratory animals is that infection can spread from animal to animal within the animal room complex. Such conditions can undermine the validity of experimental data and lead to erroneous conclusions. Such skewing can remain a variable within the experiment, or it can extend to other totally unrelated experiments. The third risk involves the ready introduction of disease into breeding colonies of laboratory animals by contamination with experimentally inoculated agents or with organisms carried by newly acquired animals. Such occurrences can have far-reaching consequences for the research of an entire institution. The costs can amount to millions of dollars in terms of loss of breeding stock and research effort. The fourth risk lies in the possibility of introducing exotic microorganisms into the environment, which in turn can infect animals of economic importance. For example, a great deal of harm can be done to the agricultural economy by inadvertently releasing microorganisms that affect livestock.

HISTORICAL PERSPECTIVE

The exact number of laboratory workers infected by contact with experimental animals is unknown. A review by Pike (1976) suggested that there is good reason to believe that animals can represent a major source of human infections. When 3,921 laboratory infections were analyzed, 659 (17

percent) were attributed to animals and their ectoparasites. This figure did not include 95 accidents *known* to involve animals or their ectoparasites. Additional infections were caused by laboratory workers inoculating themselves while attempting to bleed infected animals or in the process of inoculating them. There have been many other reports in the literature of human disease in which contact with infected animals was suspected as the cause (Parker and Spencer, 1926; Smith and Stuart-Harris, 1936; Meyer and Eddie, 1941; Lennette and Koprowski, 1943; Rowsell, 1963; *Symposium on Infections of Laboratory Animals Potentially Dangerous to Man*, 1964; Stoenner and MacLean, 1965; Baum *et al.*, 1966; Hanson *et al.*, 1967; Espana, 1971; Hull, 1973a,b; Lennette, 1973; Wedum, 1974). Phillips and Jemski (1963) estimated that animals are probably involved in 30-40 percent of laboratory-acquired infections. In one laboratory, over a period of about 21 years, the number of animal caretakers infected with organisms under investigation represented about 12 percent of the current number of employees in that category (Wedum, 1964).

The infectious hazard of laboratory animals can emanate from experimentally inoculated disease agents or from those harbored naturally by the animals. For primates, at least, the experimentally induced diseases seem to cause fewer problems than those acquired by the animals before coming to the laboratory (Gerone, 1975). Some of the so-called natural infections are really those contracted by the animals enroute to the laboratory. Nonhuman primates seem to be particularly susceptible after capture to contracting tuberculosis and hepatitis, becoming infected with pathogenic bacteria, or becoming infested with parasites (Smith *et al.*, 1967; Kissling *et al.*, 1968; Hummer, 1969; Deinhardt, 1970; Moreland, 1970; Orihel, 1970; Kalter and Heberling, 1971, 1975; Quist, 1972; Vickers, 1973; Stunkard *et al.*, 1974; Kaufmann *et al.*, 1975; Kissling, 1975).

The two most outstanding examples of contamination of laboratory breeding colonies by diseases introduced by laboratory animals are represented by ectromelia (caused by a poxvirus and characterized by gangrene and often loss of one or more of the feet and other external parts--also known as mousepox) and lymphocytic choriomeningitis (LCM). Both diseases are carried by rodents, but LCM can infect many other species, including humans as well. Ectromelia was recognized as a problem many years ago (Fairbrother and Hoyle, 1937) and it has continued to cause difficulties (Briody *et al.*, 1956; Briody, 1959; Gledhill, 1962; Schell, 1964). Most recently, the source of ectromelia contamination of lab colonies has come from the experimental use of mice imported from Europe (Whitney, 1974; Anslow *et al.*, 1975).

LCM has long been recognized as a potential contaminant of laboratory animal colonies (Armstrong and Lillie, 1934), and the subject was thoroughly reviewed by Hotchin (1971). The disease has been introduced into laboratory animals by the passage of virus-containing tumor cell lines (Taylor and MacDowell, 1949; Law and Dunn, 1951; Humphreys *et al.*, 1956; Stewart and Haas, 1956; Nadel and Haas, 1958; Haas, 1960; Traub, 1962; Jungeblut and Kodza, 1963; Lewis *et al.*, 1965). LCM also exemplifies how contaminants in animal colonies can affect the outcome of experiments. Infection of animals with this virus has been known to change susceptibility of the species to other infections and tumorigenic agents (Hotchin, 1971). Because LCM-infected animal colonies have also caused laboratory infections among research personnel (Armstrong and Dickens, 1935; Milzer and Levinson, 1942; Hayes and Hartman, 1943; Lewis *et al.*, 1965; Baum *et al.*, 1966; Armstrong *et al.*, 1969; Hotchin *et al.*, 1974), this virus can represent a triple threat to the laboratory by infecting breeding colonies, altering experimental results and causing disease in laboratory workers. The disease can also be contracted from infected monkey lice (Smith and Stuart-Harris, 1936).

A survey of the literature has not revealed any instances in which diseases of veterinary importance were introduced in domestic animal populations by infected laboratory animals. This hazard, however, must be considered at least a theoretical possibility that could have far-reaching effects. It is in recognition of this potential that facilities such as the Plum Island Animal Disease Laboratory (Callis and Cottrall, 1968) were designed and regulations (Federal Code, Title 9, Parts 104 and 122) restricting the shipment of animal pathogens were instituted. An interesting report by Sellers *et al.* (1971) demonstrated the potential of laboratory workers to transfer the virus of foot-and-mouth disease from infected to non-infected animals.

INFECTIOUS ORGANISMS USED IN RESEARCH

Virtually all organisms known to be pathogenic for humans or animals have been inoculated into laboratory hosts for experimental purposes. The spectrum of agents includes parasites, bacteria, fungi, viruses, and rickettsiae, as well as the toxic products of bacteria and fungi. In addition to the hazard of experimentally inoculated etiologic agents, a laboratory must be prepared to cope with diseases that naturally occur in research animals. The natural infections are often more hazardous than the induced type because they are often unsuspected and, therefore, go undetected until laboratory infections become manifest.

From time to time, animals infected with all groups of microorganisms have been the source of hazard in the laboratory. However, viruses, rickettsiae, and bacteria have been the chief offenders. The hazard of working with viruses has begun to receive particular emphasis because viral diseases generally are not treatable, they are harder to detect and diagnose, research on viruses has increased, and viruses may be associated with human cancer. Moreover, some of the most serious human diseases and laboratory accidents have involved viruses.

Another potential hazard may emanate from the new ability of molecular biologists to perform feats of genetic engineering. By using DNA

recombinant molecules, it is possible to introduce foreign genetic information into cells. Sooner or later, genetically altered bacteria must be tested for alterations in virulence. Such experiments, if they involve laboratory animals, would represent an unknown, but perhaps extremely hazardous, situation. For example, if such experiments were to produce strains of bacteria resistant to antibiotics, or strains capable of producing new toxins, their existence and propagation could represent a risk of entirely new proportions. Fortunately, the scientific community has taken steps to minimize the accidental release of dangerous microorganisms that might emerge from these studies (*National Institutes of Health*, 1976).

CLASSIFICATION OF HAZARDS

During the past decade, several schemes have emerged for the classification of hazardous disease agents. Since some confusion exists as to the relationship of these classifications to one another, a brief review of the essential features of each might be helpful.

The first system, developed by the Center for Disease Control (CDC), deals primarily with nononcogenic agents (U.S. Public Health Service, 1975). The National Cancer Institute (NCI) undertook the classification of the oncogenic viruses (1974). Primarily for their own use, the Oak Ridge National Laboratory (OR) prepared its own system for classifying pathogens (Lincoln *et al.*, 1970), as did Yale University for viruses (Stark, 1975). The fifth classification was published in England (Godber *et al.*, 1975).

Applicable criteria employed in the classification systems for establishing the level of risk for various etiologic agents are set forth in Table 1.

TABLE 1 Criteria for Classification of Agents as Hazardous

Originator of Classification	Criteria
CDC	Agent and its disease potential Nature and kind of study Exoticism
NCI	Potential oncogenicity for humans
OR	Pathogenicity for humans and/or animals
Yale University	Degree of known hazard to laboratory personnel
UK	Exoticism[a] Human and/or animal pathogenicity

[a]For species in Great Britain.

For comparative purposes, the risk levels within each classification system are listed in Table 2. Because of inherent differences among the classifications, it is not possible to equate levels of one system with another. It is interesting to note that, because rabies has been eradicated from England (Boulger, 1966), it is placed in the highest hazard category of the UK classification, whereas in the CDC classification, the disease is in Classes 2 and 3.

Because the CDC classification appears to be the most widely used and accepted, it is summarized in Table 3. The table also includes examples of etiologic agents assigned to the various classes, along with a brief description of recommended levels of containment.

TABLE 2 Risk Levels Within Hazard Classifications

CDC	NCI	OR	Yale Univ.	UK
Class 1 No or minimal hazard	Low Risk All not classified as moderate or high	Group A Nonpathogenic for humans and animals	Class 1 Viruses not in higher classes	Category B Agents dangerous for humans and/or animals present in England or not likely to cause epidemics
Class 2 Ordinary potential hazard	Moderate Risk Possibly oncogenic for humans	Group B Pathogenic for animals	Class II Viruses of questionable pathogenicity and those that have caused lab infections	Category A Agents capable of producing serious disease in humans and/or animals, essentially exotic to England
Class 3 Special hazard	High Risk Proven oncogenic for humans	Group C Possibly pathogenic for humans	Class III Viruses that have caused serious illness or death in lab personnel or are of unknown risk	
Class 4 Extremely hazardous		Group D Known pathogenic for humans	Class IV Viruses that pose a very high risk	
Class 5 Exotic animal pathogens				

TABLE 3 CDC Classification of Etiologic Agents on the Basis of Hazard[a]

	Class 1	Class 2	Class 3	Class 4	Class 5
Bacteria	All those not in higher classes	*Bacillus anthracis* *Staphylococcus aureus* *Streptococcus pyogenes* *Neisseria gonorrhoeae* Others	*Brucella* *Francisella tularensis* *Mycobacterium tuberculosis* Others	None	*Mycoplasma mycoides* Others
Fungi	All those not in higher classes	*Actinomycetes* *Cryptococcus neoformans* Others	*Coccidioides immitis* *Histoplasma capsulatum*	None	None
Parasites	All those not in higher classes	*Leishmania* *Toxoplasma gondii* *Trypanosoma cruzi* Others	*Schistosoma mansoni*	None	*Theileria* *Trypanosoma vivax*
Viruses, Rickettsiae, Chlamydia	PR8 Influenza Newcastle virus vaccine Others not in higher classes	Influenza Measles Mumps Polio Rabies (except in carnivores) Others	Rabies (in carnivores) Smallpox (*in vitro*) LCM Rickettsiae Others	Smallpox in animals Monkey B Hemorrhagic fever Tick-borne encephalitis Others	Hog cholera Louping ill Goat pox Rinderpest African swine fever Others
Containment Recommended	None	Ordinary microbiology laboratory	Controlled access, air barriers, special animal holding rooms	Separate building or air-handling systems, restricted access, protective clothing	Entry into U.S. forbidden by U.S. Department of Agriculture policy or law

[a]From *Classification of Etiologic Agents on the Basis of Hazard* (U.S. Public Health Service, 1975).

FACTORS THAT CONTRIBUTE TO THE HAZARD

Agent Virulence

The overall hazard that any given microorganism poses in experimental animal studies depends upon many factors. One of the most important is the virulence of the agent for the humans or animals at hand. One measures virulence by the case-fatality rate of the disease, its power to cause long-term disability, and the dose capable of producing disease. Applying these criteria, such diseases as viral encephalitis, tuberculosis, malaria, and LCM are hazardous, as all of them have been known to result in death or long-term disability. The number of microorganisms constituting an infectious dose for humans can vary considerably, depending on the organism and the route of infection (Wedum, 1964; Wedum *et al.*, 1972). Those agents that can infect with small doses by the aerosol route should be considered the most hazardous.

Transmissibility

A second factor in considering the hazards of any given agent is the probable method of transmission in the laboratory. Pathogenic organisms can be transmitted by vectors, through direct contact or by aerosols. Each mode of transmission presents a different level of hazard and each is controlled by quite different means.

Organisms such as malaria protozoa, encephalitis viruses, and the pestis bacillus (*Yersinia pestis*) that are transmissible by arthropods must be used solely in facilities in which precautions have been taken to keep the vectors away from infected animals. Once it is recognized that vector transmission is possible, the methods of dealing with this fact are relatively simple and direct.

Transmission by direct contact is a little more difficult to prevent. It is accomplished by isolating animals from one another and from the laboratory personnel. The isolation need not be airtight--rather, it is more a matter of increasing distance between the infected animals and the susceptible species. Direct contact infections caused by viruses such as rabies and monkey B can be minimized by protecting against the animals' bites and scratches.

Organisms infectious by the oral route, such as enteropathogenic bacteria, amoebae, and hepatitis virus, present still a different situation. They can infect susceptible individuals, not only in the immediate environment by hand-to-mouth contamination, but also in hosts some distance away via contaminated fomites.

In regard to accidental laboratory infections in humans and animals, the airborne route is the most hazardous. It is the most difficult mode of transmission to interrupt. Many organisms are strongly suspected of being transmitted by the airborne route in both the laboratory and the animal room. A partial list of the more virulent diseases would include tuberculosis, tularemia, Q fever, viral encephalitis, smallpox, LCM, coccidioidomycosis, histoplasmosis, anthrax, and psittacosis.

Infectious aerosols in the animal room stem

from the animals or the attending personnel. Animals can produce aerosols by sudden respiratory exhalation such as sneezing, coughing, and snorting. Rapid movement within the cage can aerosolize dust, and, with larger animals, urine splashing on drop pans could be a source of infectious hazard. Workers in animal rooms will sometimes contribute to the aerosol hazard by scraping bedding from unsterilized cages. Humans also produce aerosols when they employ high-pressure streams of water to clean cages and drop pans. Infectious dust may become airborne by dry-sweeping the floor or dusting equipment.

Finally, in any discussion of transmissibility of disease in the animal room, the extent of agent shedding by the animals must be considered. The infected animals obviously represent the ultimate source of contamination. Organisms that are shed in large quantities from skin lesions, nasal-oral secretions, or by excretions of urine and feces would pose a greater problem than those for which little or no shedding occurs. The amount of shedding is important for all routes of transmission except transmission by vectors, in which case the number of organisms present in the peripheral circulation of infected animals is of prime importance.

Prophylaxis and Therapy

If effective vaccines or immunogens are available for the agents under investigation, the hazard is drastically reduced. Both workers and laboratory animals can be protected by these immunizations. Diseases treatable by chemotherapy are usually rated as less hazardous than those that are not. Accordingly, viruses are collectively classified as a greater threat than most bacteria. However, good laboratory practice dictates that every effort be made to reduce the possibility of laboratory infections regardless of the availability of therapy. Conditions that might encourage any laboratory infections are unacceptable.

Agent Stability

The hazard of a microorganism in the laboratory and animal quarters cannot be evaluated fully unless its inherent stability is assessed. The organism capable of surviving in a wide range of temperatures and humidity and after desiccation, exposure to sunlight, and other adverse environmental conditions is obviously more resistant than highly labile organisms. Thus, control of a stable virus, such as variola (smallpox), or the spores of pathogenic bacteria or encysted protozoa must be paid special attention. Stabler organisms are more apt to survive longer in the body fluids into which they are shed, which could mean that contamination of an animal room might persist for longer periods of time. It should also be pointed out that microorganisms can show considerable variation in their resistance to disinfectants. One cannot assume that a chemical disinfectant that is highly effective against one organism will be equally useful against another.

Other Factors

Course of Infection The courses of infection in inoculated animals can have a bearing on the hazard. Acute infections of short duration from which the animal dies or recovers with full immunity represent a hazard of a different order than protracted, chronic infections. A good example of the latter is the congenital infection of mice and hamsters with LCM virus (Hotchin, 1962; Lehmann-Grube, 1971; Rowe, 1973). Such animals become lifelong carriers and shedders of the virus. Another group of chronic infections would be represented by the subacute, spongiform viral encephalopathies or slow viruses (Gajdusek and Gibbs, 1973). In addition to the risk of having infected animals in the laboratory for long periods of time, the viruses that cause the diseases are usually resistant to elevated temperatures and a variety of agents that normally deactivate most viruses. To complicate matters, it is not clear to what extent these viruses might be transmissible from animals to humans.

Size and Disposition of Animals The size and aggressiveness of infected laboratory animals bear on the magnitude of a hazard. Large animals are more difficult to isolate, and the possibility of persons becoming infected through bites and scratches increases in handling the more aggressive species. Animals that are more difficult to restrain are most likely to struggle while being handled by laboratory workers, heightening their chances of self-inoculations with needles and syringes.

Experiment Treatments Sometimes the experimental manipulations of the infected laboratory animals can add to risks incurred. For example, the immunosuppression of laboratory animals can severely alter the course of disease in the animal and might result in the reactivation of latent infections (Kirschstein *et al.*, 1961; MacCarthy and Tosolini, 1975; Vizoso, 1975).

Route of Infection The route by which laboratory animals are inoculated can influence the overall hazard of a research operation. Injections involving needles and syringes subject the worker to the possibility of self-inoculation. When inoculated intranasally, animals often produce aerosols by snorting and sneezing, particularly if they are not anesthetized. The most hazardous method of inoculation is the exposure of the entire animal to infectious aerosols. The potential for contaminated coats to infect other animals or laboratory personnel is great (Phillips *et al.*, 1956; Wedum, 1964; Kruse and Wedum, 1970; Cappucci *et al.*, 1972).

EXPERIMENTAL APPROACH TO RISK EVALUATION

It is often difficult to evaluate the hazard of working with any given etiologic agent in animals, because the literature tends to be insufficient, particularly with newly recognized agents of

disease. Under such circumstances, it becomes imperative that the researchers design experiments that will help to answer questions relevant to biohazards. If the sequence of such experiments is planned carefully, scientists can develop some sound bases for evaluating the hazard at the same time that the biological agent is being characterized.

Agent Stability

Early in the research program, experiments for determining the stability of the etiologic agent should be planned. Data on thermal inactivation, survival in a variety of laboratory disinfectants, and the susceptibility of the organism to ultraviolet light can be valuable in planning a safety program. The organism's survival in the saliva, urine, and feces of the host is particularly useful to know.

Animal Studies

Routes of Inoculation Experiments with animals should be designed to reveal routes of infection, agent shedding, and the occurrence, if any, of cross-infections. The question of route is especially important because the answer can provide some insight on how cross-infections can be avoided. If the animals are susceptible to disease by a number of routes, including the respiratory tract, the chances of cross-infection are much greater than if the disease can be introduced only by intracerebral inoculation.

Shedding of Microorganisms The investigator should know how shedding takes place and the quantities of contamination likely to be produced from it. Organisms that are shed in large quantities into the urine and feces are apt to be a main source of communicable disease. A surprising number of infectious agents known to cause laboratory infections in humans are shed in the urine and feces of contaminated animals (Wedum *et al.*, 1972).

Cross-infection Perhaps the most important criterion for defining the potential hazard of infectious organisms in laboratory animals is the extent of cross-infection that transpires. Evidence that cross-infection is prevalent would suggest the real possibility of contaminating people, colonies, experiments, and domestic animals. In experiments involving cross-infection, the researcher can investigate types of caging, route and method of inoculation, species of animals, effect of air filtration, and the use of ultraviolet light as independent variables. A dependent variable would be the amount of cross-infection that takes place.

Kruse and Wedum (1970) have made most extensive studies of cross-infection and a review of several other studies using human pathogens was published by Kirchheimer *et al.* (1961). Some other studies of experimental transmission have been conducted by Zarafonetis *et al.* (1947); Briody *et al.* (1956); Owen and Buker (1956); Rowe (1961); Alexander (1962); Parker and Reynolds (1968); Rickard *et al.* (1969); Hyslop (1970); van der Veen *et al.* (1970); Giddens *et al.* (1972); Iida (1972); and Jarrett *et al.* (1973). These studies provide good models for the design of future experiments with cross-infection.

PRECAUTIONS FOR RESEARCH ON INFECTIOUS DISEASE IN ANIMALS

There are attendant risks in virtually all the activities surrounding the use of animals in research with infectious agents. These risks begin at the time the animals are procured and will remain active in varying degrees until the animals are disposed of. In this section, an effort will be made to identify some of the hazards along the way and define some practices for reducing them.

Procurement and Quarantine

Even before animals are used for experimental work, they can represent a hazard if they are brought into the laboratory while harboring pathogenic organisms. This danger can be minimized or avoided by judicious selection of animals during the procurement process. Whenever possible, animals should be purchased from reliable commercial sources known to produce high-quality laboratory animals, and, if available, they should be specific pathogen-free. Domestically bred animals are generally safer than their feral counterparts.

The naturally occurring diseases of laboratory animals, or those that are acquired en-route to the laboratory, are often the most intractable. Natural infections are sometimes difficult to detect, because they may not manifest themselves in overt signs of disease, as is frequently the case with rodent sickness (Gledhill, 1962; Rowe *et al.*, 1963; Lehmann-Grube, 1971) and B virus in monkeys (Kirschstein *et al.*, 1961; Kalter and Heberling, 1975; MacCarthy and Tosolini, 1975). Primates are prone to contracting communicable diseases after they are captured from the wild. Many of these illnesses, such as tuberculosis and hepatitis, are of human origin and, therefore, dangerous to the laboratory worker.

Along with careful selection of animals, several measures can be taken to mitigate the risk of pathogens being introduced by incoming animals. The laboratory should quarantine newly arrived animals, particularly if the species are not domestically bred. During quarantine, animals should be observed, tested for indications of disease, and treated if necessary. Different species of laboratory animals should never be mixed in a single room, because some infectious agents can cross from one species to another.

Specific immunizations may also be used in controlling disease in laboratory animals. Mice can be immunized against ectromelia (Trentin and Ferrigno, 1957; Tuffery, 1958; Flynn, 1963; Whitney, 1974). Nonhuman

primates have been immunized against such diseases as tetanus (Digiacomo and Missakian, 1972), rabies (Richardson, 1971), yellow fever (Mason *et al.*, 1973), and smallpox and measles (Keeling and Wold, 1975). The immunization of dogs and cats to rabies and distemper is well known.

Handling and Use of Animals During Experiments

After the animals are procured and quarantined, the next step that can present hazard is the actual laboratory use of the animals. As discussed above, the procedure for initiating infections in the animals can be hazardous in itself (Grace and Mirand, 1963, 1965).

Self-inoculations are less likely to occur if animals are well restrained, either by having ample assistance for the inoculation procedure or by administering anesthesia. In both cases, the objectives are to free the individual who must inject the infectious material from having to hold the animal and to keep the animal from making sudden accident-causing movements.

Exposure of animals to aerosols containing pathogenic organisms requires special facilities to prevent airborne contamination of the work area (Wedum, 1964). If animals must be inoculated with aerosols, the possibility of contaminating other animals, and presumably workers, is greatly reduced by air-washing the exposed animals or only exposing their heads to the aerosol (Kruse and Wedum, 1970).

Once the animals are experimentally infected, they themselves become a source of laboratory contamination. This situation usually coincides with the most intensive observation period. Depending upon the experiment, it may be necessary to examine the animals at close range, draw blood, obtain biopsies, take X-rays, perform surgery, or use special monitoring equipment. Such procedures make it difficult to contain the hazard by conventional isolation techniques. Often the only practical approach is to isolate the rooms in which the animals are kept and protect all persons who must come into contact with the infected animals. Even if observations are confined to a relatively short period each day, it is still worthwhile to use isolation cages. They will help to protect attendants and other personnel exposed to the rooms for longer periods each day.

Samples that must be taken from the animal room to the laboratory for processing should be carried in airtight containers with outer surfaces that can be decontaminated. Care should be taken to disinfect the instruments, fever thermometers, and other items that might serve as fomites for the transfer of infectious materials to other animals. The same precautions should be used for gloves, whether they are of the surgical type or those worn to protect from bites. Contamination can also be spread from room to room by feet. This possibility can be avoided by providing boots that are left in the room, by using disposable foot coverings, or by instituting disinfectant footbaths.

Terminating the Experiment

The fate of most animals used in infectious disease experiments is complete recovery or death by experimental causes or euthanasia. If the animals recover, there must be some assurance that they are immune and no longer shedding disease agents. A clean bill of health is usually established through serological tests and by repeated demonstrations that the agent can no longer be isolated.

Animals that come to necropsy can still act as a source of infection. The carcasses should be transferred from the animal room to the pathology laboratory in sealed plastic bags or other airtight containers. Every effort should be made to avoid cuts or creating aerosols during the performance of a necropsy. Unused portions of the carcass should be sterilized in the autoclave or incinerated.

GENERAL MEASURES FOR COPING WITH INFECTED ANIMAL HAZARDS

In the general operation of animal facilities used for infectious disease research, certain basic practices will help to minimize the risk. Many of these have already been mentioned, because most safety rules that apply to the infectious disease laboratory also apply to the animal room.

A safety program for an animal facility should be based on concepts of preventive medicine. Protecting the environment and the validity of the experiment requires thoughtful planning about how to contain infectious agents. Although complete elimination of risk is the ideal goal, in most situations 100 percent prevention is impractical for financial or experimental reasons. In addition, for the vast majority of microbial agents used in research, complete containment of the organism is not necessary. A more reasonable alternative, therefore, is to institute hazard controls more or less consistent with the level of risk encountered.

Personnel

The proper training and instruction of personnel that come into contact with infected animals is the subject of another paper in this symposium (Vesley, 1978). People who are alert to the hazards of working with infected animals are less likely to gamble their health by exposing themselves carelessly.

Sometimes it is possible to protect personnel by prophylaxis. If good vaccines or immunizations are available for the organism under study --e.g., poliomyelitis, yellow fever, tetanus, and smallpox--immunoprophylaxis is the single most important measure for preventing laboratory infections. Animal handlers have been immunized against rabies, measles, and typhoid to protect them from diseases that might be carried by

the laboratory animals (Hummer, 1969). Gamma globulin has been administered to individuals who come into close contact with nonhuman primates that might carry hepatitis (Friedmann *et al.*, 1971).

Use of Barriers

Three sorts of barriers are used to prevent the spread of infectious disease organisms in the animal quarters. These barriers can be for rooms, cages, or personnel. Barriers in rooms are usually thought to be secondary to the primary isolation provided by the cage. Because architectural considerations are the subject of another paper in this symposium (Henke, 1978), the only point to be emphasized here is that, whenever possible, animal rooms housing infected animals should be isolated from other laboratory operations, as well as from rooms for healthy animals. They should also be located so that access to the area can be easily controlled.

Caging Even ordinary, open-top cages serve as a barrier to a certain degree because they restrict the area over which infected animals can move. With most infectious agents, such cages are sufficient to at least inhibit the spread of disease from cage to cage. However, when the open cage is inadequate for preventing microorganisms from contaminating other animals or people, then additional measures must be taken.

Strategically placed ultraviolet lights of sufficient intensity can help diminish the airborne spread of contamination in the animal room (Lurie, 1944; Phillips *et al.*, 1957; Riley, 1957; Kruse and Wedum, 1970). The disadvantage of ultraviolet light is that workers' eyes must be protected from its harmful effect.

Another arrangement that might offer some protection is an open cubicle into which standard cages are placed. The cubicle has filtration, directional airflow, and ultraviolet light to create isolation. The open-front cage with controlled airflow may be adequate for animals infected with most agents (Wedum *et al.*, 1972). This group would include low- and moderate-risk oncogenic viruses, agents belonging to CDC Classes 1, 2, and 3, and most of those in Class 4, except when the animals are exposed to infectious aerosols.

Some degree of containment within the cage can be achieved with filters and filter tops (Kraft *et al.*, 1964; Schneider and Collins, 1966; Hopkins and Drury, 1971; Burmeister and Witter, 1972; Giddens *et al.*, 1972). Filter tops are relatively easy to use in the animal room, and they are effective in preventing or reducing cage-to-cage transmission of disease. Their main drawback is that they restrict air circulation, and with some animals this can cause buildup of high concentrations of ammonia within the cage.

A cage commonly used to isolate medium-sized laboratory animals, such as monkeys, is the Horsfall-Bauer (1940) unit and its many modifications. Such cages are designed as relatively airtight units that are to be supplied with filtered air for ventilation. These units have the disadvantage of being rather expensive, and observations of the animals are difficult to make. In addition, opening the cage doors usually creates enough pressure to allow the escape of potentially contaminated air. Another technique is to place a number of standard cages in plastic or stainless steel isolators that are equipped with airlocks and filters and are maintained under negative pressure (Tauraso *et al.*, 1969).

The ultimate containment device required with hazardous organisms administered to animals in aerosols is the Class III cabinet (Wedum, 1974). These cabinets are freon-tight and operate under slight negative pressure. Unless leaks develop in the arm-length rubber gloves, the cabinets provide excellent protection. Within the cabinet, the animals are kept in more or less standard cages. The disadvantages of the Class III cabinet are many: it is prohibitively expensive, it is difficult and cumbersome to perform otherwise standard laboratory procedures in the cabinet, the gloves are prone to develop leaks, and it is difficult to move scientific equipment in and out of the system.

Isolation cages are impractical for larger animals such as pigs, goats, sheep, and cows. If it is necessary to isolate these animals, it is generally accomplished in a room. Workers must be protected when entering these infected areas. Large animals have been isolated out-of-doors by space dilution, which can be employed when the research concerns diseases that are not infectious for humans. To accomplish this isolation, widely separated paddocks are erected in areas where susceptible species are not free-ranging. Before space dilution can be used, however, investigators must furnish reasonable assurance that wild animals and birds found in the area are not susceptible to the infectious agent.

Personnel Protection Depending on the degree of primary isolation in an animal room, workers can wear several protective items that will serve as barriers to the etiologic agents, e.g., clothing such as autoclavable cotton or disposable surgical gowns and caps. It may be desirable to wear foot coverings, which can remain in the infected animal room or be incinerated. Since it is relatively common for workers to have minor cuts and abrasions about the hands, it is advisable for them to wear surgical gloves. Heavier gloves to protect from animal bites may also be necessary.

Other protective devices needed in the animal room include respirators and eye shields or goggles. If any possibility exists that the organisms under study or those that are carried naturally by laboratory animals can infect humans by the airborne route, it is imperative that a good-quality respirator be worn. Surgical-type cotton or disposable masks do little to protect the individual from small-particle

aerosols. In working around such pathogens as adenoviruses, Newcastle disease virus, and monkey B virus, all of which have the potential to infect by the occular route, wearing eye protectors is highly recommended. When the airborne hazard is extremely high, such as when workers are around animals infected with extremely dangerous human pathogens, they may have to be protected with airflow personnel hoods (Wedum *et al.*, 1972) or plastic space-type suits with a filtered air supply.

CONTROLLING AIRBORNE CONTAMINATION

Any operation or activity tending to generate aerosols in a room with infected animals is best avoided. Some sources of aerosols are the stirring up of dust from contaminated cage bedding, the disturbance of contaminated fur or feathers, and the use of high-pressure hoses to clean drop pans and cages. Drop pans placed too far from the bottom of the cage could create aerosols through the splashing of urine. Sweeping dry floors and dry-dusting cage racks and other animal room equipment may also produce aerosols.

DECONTAMINATION

From time to time it is necessary to decontaminate cages and other equipment that have had contact with infected animals found in the animal room. Decontamination is most effectively done by autoclaving. Dirty cages with bedding should be sterilized before an attempt is made to remove bedding material. If cages are too large, or are permanently attached to the wall, it is not possible to use the autoclave for sterilizing them. Under these conditions it is necessary to use chemical disinfectants known to inactivate the infectious agents in question. The disinfectants are usually applied with some form of spraying device. Some of the more commonly used disinfectants are halogens, phenolics, and quaternary ammonium compounds. (Glutaraldehyde is becoming established as an excellent new germicide.)

Sensitive scientific equipment that becomes contaminated in the animal room can be sterilized with gaseous disinfectants. The most commonly used gas is ethylene oxide. Ethylene oxide can be introduced into an autoclave that is equipped to accept it, or into a sealed plastic bag containing the equipment to be sterilized. The latter method provides considerable flexibility in sterilizing a variety of items.

The animal room and its contents can be decontaminated with a formaldehyde gas, most easily produced with paraformaldehyde. The room is sealed and the paraformaldehyde is depolymerized in an electric frying pan. The temperature of the frying pan is set at 232°C. For each cubic meter of room volume, 10 g of paraformaldehyde is required. The relative humidity of the room should be maintained at about 60 percent. After about 1 hour, the room is ventilated to remove the formaldehyde gas.

SUMMARY

It is possible for us to work safely with even the most hazardous disease agents in animals. Most laboratories certainly have the technological capability to experiment with low- and moderate-risk agents in laboratory animals. Thus, the difficulty lies in developing a safe operation suitable to the risks likely to be encountered. A safety program must match the hazard, because, if overdone, it becomes too costly and time-consuming. If it is too lax, serious consequences may result. Deciding which safety measures to employ with any given host-parasite combination becomes relatively simple when reliable empirical information on the hazard is available. The first step, therefore, is to gather data while taking all possible precautions. Then, using common sense and conclusions based upon those findings, institute measures that will minimize the hazard.

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Chemicals and Toxins in the Animal Facility

PAUL M. NEWBERNE and JAMES G. FOX

DEFINITION, CLASSIFICATION, AND ROUTES OF TOXIC SUBSTANCES

Chemicals and toxins can pose a threat, real or potential, to the health and welfare of laboratory animals; the safe use of such substances must receive prime consideration in the planning of the total laboratory animal environment. Not only may their misuse compromise the animals but it may endanger animal-care personnel and investigators as well. Some dangerous chemicals may be necessary to the experimental environment, such as those used to control pests and diseases or ascertain an agent's hazards to an animal. If the laboratory staff strictly adheres to good laboratory practices and follows the recommendations for a chemical's use, those useful compounds and necessary tests are not likely to pose a danger. Managing the unintentional introduction of chemicals and toxins into the animal facility is, however, more difficult. Whether contamination is a consequence of human activities (e.g., lead in feeds, spilled insecticides, carcinogen feeding studies, etc.) or a natural phenomenon (e.g., mycotoxins in feeds), the outcome is the same: outright loss of animals or, even more insidious, biased interpretation of results.

The term "toxic substances" includes all mined, manufactured, processed, synthesized, and naturally occurring inorganic and organic compounds. This list of approximately 100,000 toxic substances encompasses a myriad of compounds, such as acids, detergents, soaps, drugs, and pesticides, all of which are routinely encountered in the animal facility. Illustrating the danger of exposure to toxic chemicals in the laboratory, Li *et al.* (1969) found a higher proportion of deaths from cancer among chemists than among other professionals. Although laboratory workers probably face much less exposure to various chemical agents than do some industrial workers, those of us who must handle potentially hazardous substances in the animal laboratory cannot be complacent; we must act to minimize exposure and risk to personnel in the facility as well as to the animals housed there. A hazard is present whenever an injury can easily result from the nonprescribed use of a chemical. This potential danger emphasizes the necessity for identifying toxic agents in the environment, formulating safety guidelines, and adhering to safety practices (listed in Table 1) for toxic substances used in the animal laboratory.

In general, a chemical can be categorized as a toxicant irritant, nuisance, carcinogen, or narcotic. These designations quickly acquaint the users of such substances with their basic properties and potential hazards. Other criteria helpful in defining the degree of risk to humans and animals are the physical characteristics of the substance, its interactions with other substances in the laboratory, and the physical environment in which exposure to the substance occurs. All these factors may greatly influence the toxic potency of an agent and, hence, also affect the health or behavior of the person or animal exposed to it.

To assist in evaluating the risk of long-term exposure to a toxic substance, scientists have established threshold limit values (TLV's) for many chemicals; these specific concentrations were determined by the American Conference of Government Industrial Hygienists and, subse-

TABLE 1 Safety Practices for Storage and Handling of Hazardous Chemicals

1. Identify and label all hazardous chemicals by affixing a "Biohazard Warning" label to them.
2. Store explosive chemicals in explosive-proof containers.
3. Provide safety equipment (protective clothing, respirators, showers, eye baths, etc.) for personnel handling hazardous substances.
4. Provide adequate training of personnel on hazards and safety protocol.
5. Store volatile chemicals so vapors cannot collect and create a hazard.
6. Maintain a current inventory of chemicals and store them under conditions that ensure stability.
7. Establish standard procedures for handling spills.
8. Limit access to the storage area.

quently, were spelled out in the Occupational Safety and Health Act of 1970 (OSHA). Each TLV represents time-weighted averages reflecting the environmental conditions under which workers may repeatedly be exposed without adverse effect.* The TLV is only one of the values listed in the *Registry of Toxic Effects of Chemical Substances*, published annually by the U.S. Department of Health, Education, and Welfare (Christensen and Luginbuhl, 1975). This collection of information, a valuable guide to toxic substances and their effects, is based on measurements of known doses entering the body by many different routes. A listing of suspected carcinogens is also available from the same agency (Christensen *et al.*, 1975).

The sources of chemicals and toxins in the animal facility are, of course, numerous. Many potentially hazardous substances can simply enter the animal facility via the air, water supply, animal food, or bedding, as elucidated in Table 2. In many instances, researchers intentionally bring the substance into the animal facility, either to control the environment (e.g., with insecticides, detergents, and disinfectants) or supplement the experimental design (e.g., with anesthetics, drugs, and carcinogens).

Stokinger (1967) has documented the several routes by which toxic agents may enter the bodies of humans and animals. The skin, because of its thickness, its keratin layer, and its film of lipid and sweat, serves as a major biological defense against such exposure. However, the skin may react to the toxic agent and become irritated and inflamed; some agents even have the ability to penetrate intact skin, react with tissue protein, and create allergic sensitization or acute or chronic systemic effects. The skin has varying concentrations of enzymes capable of metabolizing chemicals to more or less toxic forms. Protective clothing and gloves can be an important barrier against contamination via the skin.

*For 8 hours a day, 5 days a week, for their working lifetime.

TABLE 2 Chemical Substances Commonly Found in the Animal Facility

Source of Contaminant	Type of Contaminant
Organic solvents	Ethers, alcohols, chloroform, carbon tetrachloride, acetone
Air	Dust and bedding particles, irradiation, trace volatile anesthetics, animal room deodorants (volatile hydrocarbons), disinfectant sprays (eucalyptol), pheromones, vinyl chloride, ammonia, insecticides, piperonyl butoxide
Diet	Nitrates, cadmium, arsenic, lead, aluminum, mercury, nickel, insecticides, mycotoxins, herbicides, chloroform, food additives, estrogenic compounds, polycyclic hydrocarbons, phenothiazines, phenylthiazoles, flavones, antibiotics
Bedding, caging, and equipment	Detergents, disinfectants, soaps, acids, ethylene oxide, wood alkaloids, cedrene, cedrol, ammonia, lignin aldehydes, antibiotics, microbiocides
Dosing and treatment of animals	Mutagens, teratogens, carcinogens, toxic agents, drugs, vaccines

Inhalation is the most common route by which injurious substances, in the form of volatile or particulate matter, can enter the body. The size and surface area of aerosolized, toxic particulate matter are important in determining rates of exposure and subsequent tissue damage. Particles of 10 μm or less in diameter, which easily form stable suspensions in the air, can travel by air currents and contaminate equipment and clothing; a particle of this size can be inhaled, and, if its size is 5 μm or less, it may penetrate the pulmonary alveolar wall (Landahl, 1963; Hatch and Gross, 1964). Respirators and safety masks can help to prevent this type of contamination.

Ingestion of injurious agents is much less common. Proper safety precautions and the training of the animal technicians can virtually eliminate the spread of toxic agents to food, hands, or cigarettes. A substance of larger particle size may, however, be inhaled, caught in the upper respiratory tract, swept up by ciliary action, and subsequently swallowed.

In this paper, we describe the main physical areas of laboratory activity that may employ toxic substances. These categories of activity, diagramed in Figure 1, encompass all major portions of the animal facility environment. We will review each work area separately, identify the major toxic substance, and assess its hazard in relation to personnel and/or animals.

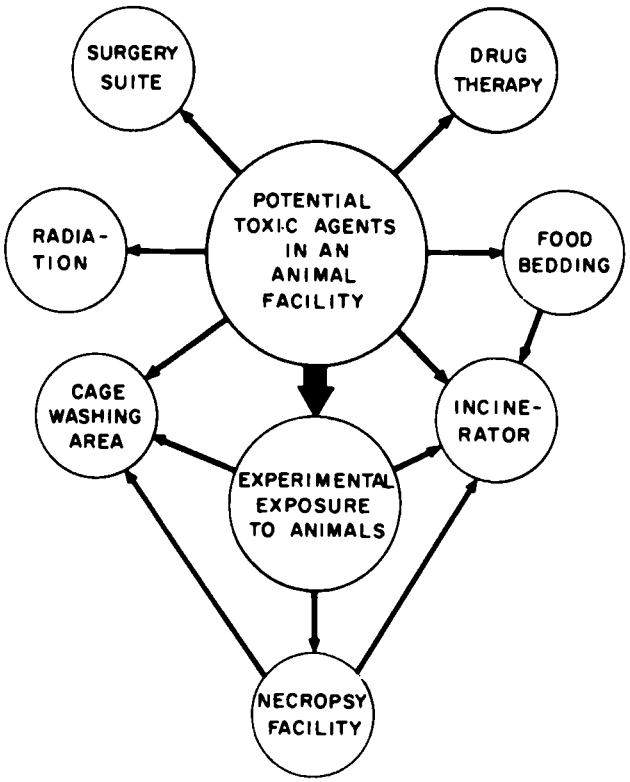

FIGURE 1 Sources of potential exposure to chemicals and toxins in the animal facility.

THE USE OF VOLATILE ANESTHETICS IN THE SURGERY ROOM

The increasing use of inhalation anesthetics in recent years has raised questions on the potential occupational health hazard of breathing the expired anesthetic gases present in the operating room. As early as 1900, German surgeons complained about phosgene produced from chloroform vapors heated by gas lamps in the surgery suite. They claimed that ether caused headaches, fatigue, and irritability in the surgery teams. Investigators used to anesthetize small laboratory animals with ether. Divinyl ether, isopropyl ether, and other alkyl ethers tend to absorb and react with oxygen from the air to form unstable peroxides, which are extremely explosive when concentrated by evaporation or distillation. Sebesteny (1971) attributed serious laboratory fires to the use of ether during animal experiments. The most common sources of ignition are electrical equipment, hot surfaces, sparks, static charges, and friction. Basic laboratory safety requires the proper placement of switches and electrical grounding and the adherence to fundamental rules that can minimize the formation of peroxides in ethers. These measures are summarized in Table 3. Because of its many undesirable characteristics, such as the narrow margin of safety and toxicity to the liver, ether as an anesthetic agent has decreased in popularity since the discovery of nonflammable anesthetics. Chloroform is no longer advocated as an anesthetic agent because of its potential toxic and carcinogenic effects (Drill, 1952; National Cancer Institute, 1976).

Information generated over the past 12 years indicates that commonly used anesthetic agents--methoxyflurane, halothane, and nitrous oxide--are a potential health hazard. The discovery of occupationally rooted diseases often stems from the identification of maladies among individuals engaged in the same career and the subsequent determination of the offending agent in the workplace. A survey of 303 Russian anesthesiologists showed a high incidence of headaches, fatigue, irritability, nausea, and pruritus (Vaisman, 1967). Spontaneous abortions were also noted in 18 of 31 pregnancies. The following year, a report of deaths among anesthesiologists over a 20-year period revealed a higher than normal incidence of reticuloendothelial and lymphoid malignancies (Bruce *et al.*, 1968). Other data have demonstrated that miscarriage, spontaneous abortion, congenital anomalies in offspring, and malignancy occur more frequently than normal among women exposed to volatile anesthetics (Cohen *et al.*, 1971; Knill-Jones, 1972; Corbett *et al.*, 1973). Linde and Bruce (1969) were the first to document overt exposure to anesthetics by recording concentrations of 27 ppm halothane and 428 ppm nitrous oxide in the surgery room; Askrog and Peterson (1970) later recorded average concentrations of 85 ppm halothane and 7,000 ppm nitrous oxide in the inhalation zone of the anesthetist when a noncirculating gas apparatus was used. Methoxyflurane concentrations of 2-10 ppm around the anesthetist and 1-2 ppm around the surgeon have also been recorded (Corbett and Bull, 1971).

A national study of occupational disease among operating-room workers compared 49,585 exposed personnel to 23,911 unexposed controls. The results indicated that women in the operating area were subject to increased risks of cancer, hepatic and renal disease, spontaneous abortion, and congenital malformations in their offspring (Ad Hoc Committee of the American Society of

TABLE 3 Protection Against Hazards from Peroxides in Ether[a]

1.	Test for peroxides in open containers of ethers and dispose of containers with high concentrations.
2.	Purchase ethers in the smallest practical size and stipulate their packaging in iron containers.
3.	Establish time limits for the storage of opened containers.
4.	Label containers as to date received and date opened.
5.	Purchase refrigerators or cooling equipment that meet specific requirements for storing ether.

[a]From Steere (1967a).

Anesthesiologists, 1974). Acknowledging the shortcomings of a survey conducted by questionnaires, the Committee concluded that the most reasonable explanation for the differences was exposure to waste anesthetic gases among the operating-room personnel. Walts *et al.* (1975) criticized the study, citing deficiencies in the statistical analysis and logic used to derive the conclusion; a reply to this criticism, however, elaborated on the statistical analysis and pointed out that the Committee had submitted the results to many experts to determine if the data supported the reported conclusions (Cohen and Brown, 1975).

This debate may well continue; nevertheless, others have found that anesthesiologists may develop sensitivities to small doses of halothane that are manifested clinically as jaundice or abnormal liver function (Belfrage *et al.*, 1966; Klatskin and Kimberg, 1969). Facial acneiform dermatitis has also been seen among anesthesiologists using halothane (Soper *et al.*, 1973). Animal studies have demonstrated the teratogenic effects of inhaling high concentrations of anesthetics (Smith *et al.*, 1965). Yet rats exposed to 100 ppm halothane daily for 8 months had no pathological lesions (Linde and Bruce, 1968), nor were toxic effects on pregnancy demonstrated from low concentrations of halothane inhaled by mice or methoxyflurane breathed by rats (Bruce, 1973). Research on miniature swine demonstrated that liver metabolism of halothane occurs predominantly at subanesthetic doses (0.0006 percent). From this finding, Sawyer *et al.* (1970) postulated that, because of chronic exposure to low concentrations of halothane, anesthesiologists may produce larger quantities of metabolites than do patients. An anesthetist using radioactively labeled halothane to study biotransformation found that he excreted twice as many radioactive metabolites as an associate who was not an anesthesiologist (Cascorbi *et al.*, 1970). Others have exposed rodents to subanesthetic doses of halothane for 35 days and demonstrated hepatotoxic lesions (Stevens *et al.*, 1975). Halothane has also produced liver lesions in guinea pigs (Hughes and Lang, 1972), and animals receiving anesthetics evidenced immunosuppression (Bruce and Wingard, 1971).

Epidemiological studies in humans, the documentation of halothane sensitivities in anesthetists, and the confirmatory experiments in laboratory animals strongly support the view that protective measures must be incorporated into operating-room procedures to minimize exposure to volatile anesthetics. Safety scavenger systems in which gas traps are placed over the pop-off valve or exhaust systems that shunt waste gases out of the operating room have strikingly reduced atmospheric contamination from halothane or methoxyflurane (Whitacher *et al.*, 1971). Such equipment should be added to all existing animal operating rooms and be included in any future designs. Connecting the pop-off valve to the exhaust of a noncirculating air conditioning system can remove up to 95 percent of the anesthetics. Scavengers that connect the exhalation portion of a closed anesthetic system to the suction equipment, which drains to the outside, also help to eliminate 90-95 percent of the nonexplosive anesthetics, as shown in Figure 2. An additional safeguard would be the use of leak-proof anesthetic machines with minimal airflows and, when feasible, closed systems. Simple and accurate gauges should be checked routinely to detect waste anesthetics. Women in the first 3 months of pregnancy must avoid contaminated surgery rooms (Whitacher, 1974).

DISPOSAL AND CLEANING SYSTEMS

The cage-washing and incinerator areas receive all the contaminated equipment, bedding, and other wastes from the animal population. Cleaning the cages usually entails the application of detergents, disinfectants, and acids to caging material. An inspection of the floor surrounding the cage-washing area in many facilities will attest to the caustic nature of those agents. Residual amounts of these products may inadvertently irritate the skin of personnel working in the area or animals that come in contact with chemicals left on caging or instruments. Padnos *et al.* (1965) have reported cases of dermatitis resulting from an antiseptic, benzalkonium chloride, applied to human mucous membranes. Mice suffered death and severe skin lesions from exposure to an improperly diluted solution of disinfectant (again, benzalkonium chloride), which was accidentally transferred to the mice from contaminated forceps (Serrano, 1972). Toxicosis from benzalkonium chloride caused hypersalivation, vomiting, central nervous system depression, and dermatitis of the feet in laboratory dogs (Grier, 1967); a 1 percent solution of the disinfectant had leaked into the dog run, and the animals poisoned themselves by licking the chemical from their inflamed feet. One report documented the loss of large groups of turkeys and chickens through their intake of quaternary ammonium disinfectants in the drinking water (Reuber *et al.*, 1970). Disinfectants containing phenol are also extremely poisonous, particularly to cats (Ernst *et al.*, 1961).

In research with gnotobiotes, equipment and supplies are sterilized with peracetic acid, and special protective clothing, gas masks, and gloves must be worn to prevent irritation to mucous membranes (Pleasants, 1974). The mask must cover the eyes *and* the nose.

For cleaning contaminated animal cages, one should take special care to select an adequate cage washer. Animal cage washers are available in three forms: those that fill and dump fresh water for each cycle of operation, those that recycle the wash water from storage tanks, and those that replace the wash water with rinse water (Ament, 1971). Cage washers that reuse the rinse or wash water can recontaminate cages with carcinogens or other toxic substances not effectively removed from the equipment surfaces. In one study (to be described later in more

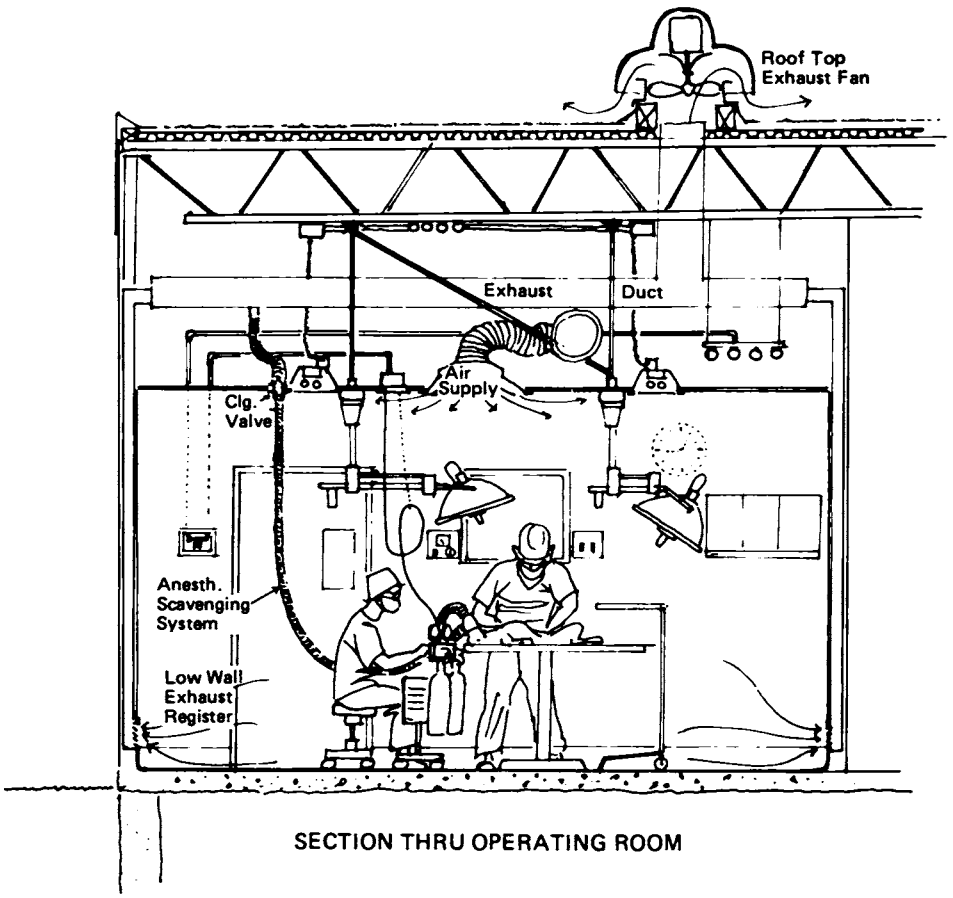

FIGURE 2 Exhaust system in operating suite, with scavenger apparatus attached to vent of gas anesthesia machine.

detail), sodium fluorescein, simulating a carcinogen, was mixed at a concentration of 3,000 ppm in a powdered-meal diet and fed to rats. The later washing of the cages did not completely remove the tracer material from the dirty, solid-bottom rat cages. The cage washer recirculated the last cycle of rinse water to be used for the first wash cycle (Sansone *et al.*, 1977). In addition, some of the cages emerged from the wash with debris still encrusted on them.

By radioactive labeling of two carcinogens, Sansone *et al.* (1977) determined the degree to which such substances could be removed from tissue-culture cells that had been contaminated with the carcinogens. Repeated washings with methanol and a balanced salt solution did not totally remove the labeled material from the cells or dishes, and the authors recommended that the dishes be handled at all stages as though they were contaminated with hazardous chemicals. The findings of this study may be compared to data cited for the washed, but still contaminated, cages. Whenever animal caging has been heavily contaminated with carcinogenic, mutagenic, teratogenic, or other toxic substances, the laboratory personnel should handle these cages with gloves at all times; they should also use disposable cages or, as an alternative, not mix reusable cages with cages from other toxicity studies or with equipment used with the general animal population.

Laboratory workers must also ensure the removal of disinfectants and detergents or solvents by thoroughly rinsing cage surfaces. Such cleansing is important because the microsomal hepatic enzyme system of the rodent is inhibited in the presence of organic solvents, cleansing substances, and buildup of cage ammonia (Weatherby, 1952; Saz and Marmus, 1953; Vesell *et al.*, 1973; Wheeler *et al.*, 1975; Vesell *et al.*, 1976).

Incineration remains the most economical method by which to destroy toxic or carcinogenic material that has contaminated animal bedding and wastes. Sufficiently high temperatures can reduce many of these compounds to harmless chemicals. Liquid chemicals and gaseous chemicals with substantial vapor pressure require special handling: One should dispose of them in incinerators equipped with scrubbers, filters, and flammable liquid tanks to meet stringent Environmental Protection Agency (EPA) standards (Larkin, 1963; Environmental Protection Agency, 1971, 1973, 1975). Other common methods of waste disposal include evaporation, neutralization, and dilution. In all instances of disposal for toxic agents, local, state, and federal guidelines must be followed (Gaston, 1964; Steere, 1967b).

The disposal of dead animals should conform to standards and procedures established for other contaminated material. Several agents, depending on the size and species of the animal, have been approved for the painless killing of laboratory

animals (Report of the American Veterinary Medical Association Panel on Euthanasia, 1972). The use of chloroform is to be avoided because of its potential danger to humans and because of the obvious discomfort it causes the animals, as manifested by their salivation and excitement (Clifford, 1971). When using volatile agents such as ether, the laboratory worker should sacrifice the animal under a properly ventilated fume hood. In addition, flammable vapors, originating from animals killed with ether, can create a hazard if the carcasses are placed in an incinerator or an improperly desigend refrigerator (McIntyre, 1971). In one incident witnessed by the authors, an overzealous graduate student put recently etherized rat bodies in the incinerator; the resulting explosion blew off the incinerator lid. Fortunately, no one was harmed. Carbon dioxide gas flushed into a closed chamber offers a satisfactory, acceptable form of euthanasia and avoids the risks associated with other volatile compounds.

Autopsies of animals contaminated with toxic substances should be performed on tables designed with plumbing and fume hoods for directing and removing toxic substances. The prosector must wear protective clothing, gloves, and face mask. We encourage the use of safety cabinets if the toxic substance is highly hazardous.

ANIMAL FOOD AND BEDDING

Many investigators have discovered that the immediate environment of the laboratory animal markedly influences its biological response to experimental manipulation. In this symposium, Lindsey *et al.* (1978) have documented the biological responses of animals to various physical and chemical factors. A pertinent example is the biological variation in a rodent's hepatic microsomal enzymes when it is housed on cedarwood or softwood bedding rather than hardwood bedding (Ferguson, 1966; Vesell, 1967), or housed in a clean environment rather than a dirty one (Vesell *et al.*, 1973, 1976). The finding that the *p*-*o*-methyl derivatives of sinapaldehyde and 2,6-dimethoxy-1, 4-benzoquinone are carcinogenic for the rat suggests that the β-saturated carbonyl compounds in wood shavings are potentially carcinogenic (Schoenatal, 1973). Indeed, workers exposed to fine particulates of hardwood, or to its volatile products generated during machine processing, develop a high incidence of nasal tumors (Acheson *et al.*, 1968). In addition, evidence now indicates that innocuous animal-room deodorizing agents, which contain volatile hydrocarbons (Cinti *et al.*, 1976) or disinfecting sprays containing oils and vinyl chloride (Jori *et al.*, 1969; Vesell *et al.*, 1976), are capable of inducing or inhibiting hepatic microsomal mixed-function oxidase systems in laboratory animals.

Several reviews and articles have been published on carcinogenic and teratogenic effects of insecticides in animals and acute toxic manifestations of poisoning in humans (Hamilton and Hardy, 1974; Vettorazzi, 1975; Aldrich and Gooding, 1976). Insecticides commonly used in animal facilities--particularly the chlorinated hydrocarbons--are potent inducers of hepatic microsomal enzymes in rodents (Kolmodin *et al.*, 1969; Poland *et al.*, 1970). Several reports demonstrate the deleterious effects of insecticides on the immune system. Dipping mice in a miticide solution brought on acute lymphocytopenia and leukocytopenia (Keast and Coales, 1967). After chronic administration of 200 ppm of DDT in their drinking water, the mean antiovalbumin titers in rats fell 30 percent and serum immunoglobulin levels were generally depressed (Wassermann *et al.*, 1969). Ambrose and Bennett (1977) showed that an organophosphate insecticide (Diazinon) and an insecticide synergist (piperonyl butoxide) inhibited the secondary antibody response in rabbit lymph-node cultures; moreover, their *in vivo* experiments with different strains of mice demonstrated a depression in the immune response when three different commercial insecticides (two containing Diazinon) were sprayed into the animals' cages.

Unwanted variables in the diet--i.e., the presence of chemicals and extraneous material or variations in the concentrations of essential nutrients--can markedly influence the biological response of animals and thus alter the interpretation of experimental data. Analyses conducted over the past 17 years on standard, commercially prepared diets for rats have disclosed widely variable concentrations, not only of essential nutrients, but also of biologically active contaminants in the food mixtures (Newberne, 1975). Findings from these comparisons are summarized in Table 4.

Because no biological requirement for some trace elements (e.g., lead, mercury, arsenic, and cadmium) has been discovered to date, one may consider these elements xenobiotics that present a hazard directly proportional to their body burden. Analysis of lead content in 103 samples of laboratory animal, dog, and cat foods revealed a range of 0.1-7.6 μg of lead per gram of food (Fox *et al.*, 1976), as shown in Table 5. In addition, 114 specific food ingredients were analyzed from 5 major commercial makers of animal foods. The lead content in each particular food ingredient varied from 0.1 to 3,600 μg of lead per gram of food. The mineral mix was consistently contaminated with high concentrations of lead (Fox and Boylen, 1978). In humans, as in other animals, lead makes its toxic effects felt on several target organs, including the nervous system, kidneys, and the erythropoietic system. Indeed, by inhibiting some enzyme systems, lead exerts widespread biological effects. Some of these changes are subtle: The metal has been shown to reduce the resistance of mice to bacterial infections and reduce antibody formation (Hemphill *et al.*, 1971; Koller and Kovacic, 1974); it also increases manyfold the susceptibility of rats and chickens to the effects of bacterial endotoxins (Selye *et al.*, 1966; Truscott, 1970; Trejo *et al.*, 1972) and suppresses immune response to pseudorabies virus in rabbits (Koller, 1973). Two other endogenous disorders common in laboratory animals--anemia and dimin-

TABLE 4 Content of Selected Components in Randomly Sampled Natural-Product Diets for Rats[a]

Component	Analysis[b]			Requirements of Rats[c]
Calcium, %	0.5	0.27	0.89	0.56
	1.67	0.82	2.10	0.44
Phosphorus, %	0.20	0.79	0.45	0.44
	1.90	0.68	0.13	
Selenium, mg/kg	0.002	0.007	0.17	0.04
	0.05	0.21	0.001	
Iodine, mg/kg	0.10	0.73	1.67	0.17
	0.08	1.35	2.00	
Tryptophan, %	0.10	0.39	0.08	0.17
Methionine, %	0.13	1.21	0.22	0.67
	0.75	0.18	0.40	
Vitamin A, mg/ retinol/kg	0.28	0.90	0.50	0.67
	2.10	0.31	3.75	
Vitamin D, IU/kg	987	1,360	5,100	1,111
	3,700	650	2,400	
Aflatoxin, ppm	0.04	0.20	0.12	
DDT, ppm	0.17	5.0	--[d]	
	2.1	0.0	0.0	
Nitrates, ppm	0	23	3	
	90	5	0	
Lead, ppm	0.80	1.90	8.50	
Cadmium, ppm	0.11	0.47	0.87	

[a]From Newberne (1975). Reprinted from *Federation Proceedings* 34:209-218.
[b]Each row across represents lots of a single rat diet product from one manufacturer; different rows for the same component represent different manufacturers.
[c]Based on the NAS recommended levels for rats (Board on Agriculture and Renewable Resources, 1972).
[d]Information unavailable.

ished renal function--can cause liver disease and, also, can alter the animals' response to steady, low-level, lead ingestion. Cadmium, also present in commercially prepared diets, and lead have been shown to decrease the life-span of rats (Schroeder *et al.*, 1965). Cadmium and mercury fed to laboratory animals produce immunosuppression (Koller, 1973). When administered to animals over long periods, cadmium is known to produce tumors. Arsenic has been strongly implicated as a carcinogen for people, but its carcinogenicity has not been proved for animals (Cole and Goldman, 1975). Other trace metals that are suspected carcinogens are found as residues in foods; they include chromium, cobalt, selenium, and titanium (Underwood, 1971; Wolff and Oehme, 1974).

Mycotoxins have existed as contaminants of food for many centuries (Burnside *et al.*, 1957); their full importance was not recognized, however, until Asplin and Carnaghan (1961) defined a toxicological disease in turkeys and identified the etiologic agent as a mycotoxin. Aflatoxins, identified in peanutmeal in the United States, constitute a source of acute and chronic disease in animals (Newberne *et al.*, 1964). Other studies have demonstrated that many food products in the United States, such as corn, wheat, and other cereals, are subject to contamination from aflatoxins (Wogan, 1968; Lillehoj *et al.*, 1976).

The acute toxicity of nitrate ingestion in animals is also well known (McIlwain and Schipper, 1963). Nitrate's ability to react with amines to form nitrosamines, many of which are potent carcinogens, renders the presence of these compounds in foods an important variable that should be considered by those interpreting data on animals fed commercial chow (Magee and Barnes, 1967). In studies of teratology, mutagenesis, or carcinogenesis in animals, fumigation of animal food with ethylene oxide can significantly reduce concentrations of vitamins and proteins and therefore alter experimental results (Bakerman *et al.*, 1956; Windmueller *et al.*, 1959).

When chemicals are incorporated into a diet

TABLE 5 Lead Content of Animal Foods by Company[a]

Species	Number of Samples	Lead content, ppm		
		Company 1	Company 2	Company 3
Rat	5	0.1 0.8	1.1 0.5 1.5	
Monkey	3	1.0 1.0	0.2	
Cat	4	4.0 1.8 3.2	3.7	
Rabbit	3	1.5	1.9 1.3	
Dog	4	2.9 3.4	1.3 1.6	
Guinea pig	3	0.9	1.1 1.2	
Pigeon	1			0.9
Monogastric animal	1			1.8
Livestock	1			0.8

[a]From Fox *et al.*(1976).

(as in toxicology studies) and when vehicle solvents such as ether or acetone are used in preparing that diet, these solutions can be highly flammable; consequently, heat from the mixing process may initiate an explosion (Robinson and Emerson, 1972). Hence, this activity should be carried out in safety laboratories with properly designed mixers. Adequate control diets (containing only the solvents) can ensure against misinterpretation of data caused by toxic effects of solvents in experimentally dosed animals.

Many natural products found in food--such as flavones--are associated with the induction of drug-metabolizing enzymes into the intestine and other tissues (Wattenberg *et al.*, 1968). When studies with laboratory animals use natural rather than semisynthetic diets, often microsomal enzyme activity increases and tumor incidence decreases. The presence of insecticides, chlorinated hydrocarbons, polycyclic hydrocarbons, phenothiazines, and phenylthiazoles in food can heighten microsomal enzyme activity in the liver. Another persistent pesticide, hexachlorobenzene (HCB), has been recovered from commercial monkey food in concentrations great enough to alter significantly the interpretation of experimental results (Yang *et al.*, 1976). Among some 30 batches of analyzed monkey chow, the HCB content ranged from less than 11 ppb to 21.1 ppm. Barsotti and Allen (1975) documented a hazardous effect from consumption of polychlorinated biphenyls: At dietary concentrations lower than or equal to the "safe" amount set by the Food and Drug Administration for human consumption, these substances affected the pregnancies of rhesus monkeys.

Hence, it is advisable for investigators conducting long-term studies on animals to observe the progress of pesticides, mycotoxins, and trace minerals in each batch of animal diet in view of the biological consequences they may have for the experimental regimes. Many additional real or potential toxicants are known to occur naturally in plants and plant products; in addition, substances may be incorporated into animal feeds (Boyd and Shapleigh, 1954). These substances include toxic proteins and peptides, compounds in favism (Liener, 1966), vasoactive and psychoactive substances (Udenfriend and Zaltman-Nirenberg, 1963; Hodge *et al.*, 1964; Blackwell, *et al.*, 1967), antivitamins (Somogyi, 1973), enzyme inhibitors (Feeney *et al.*, 1969), and estrogenic substances (East, 1955).

Potentially toxic contaminants also are found in tap water, and their presence should be considered when one performs long-term animal studies that utilize municipal-grade water. According to EPA reports, chloroform is common in municipal water sources. Other compounds, sometimes found in water, with known or suspected carcinogenic ability are carbon tetrachloride, polychlorinated biphenyls, benzene, benzo[*a*]pyrene, trichloroethylene, *bis*(2-chloroethyl) ether, and diphenylhydrazine. This is merely a partial list of organic contaminants in drinking water: Although studies have identified approximately 90 percent of the volatile organics that are present, these contaminants represent only 10 percent of the total organics found in water supplies (Safe Drinking Water Committee, 1977).

THERAPY

Researchers frequently administer such drugs as antibiotics to laboratory animals, especially the large unconditioned animals (e.g., dogs, cats, nonhuman primates); in particular, antibiotics are used to treat postoperative infections. Their acute side effects have been well documented through toxicity studies and clinical trials; because unintended toxic reactions are relatively common, the clinician must be familiar with any potential untoward reaction. Recent studies have demonstrated that, under prescribed conditions, antibiotics (lincomycin, tetracycline, vincomycin, streptomycin, and other aminoglycosides) can cause cardiovascular depression that would alter experimental results (Adams, 1975a,b). The clinical cardiovascular manifestations of these various antibiotics are decreased cardiac output, hypotension, and, in some cases, arrhythmia and decreased heart rate.

The interaction of various drugs and injectable anesthetics can influence depth of anesthesia and recovery and the phenomenon is clinically important. For example, therapeutic doses of chloramphenicol prolong the duration of pentobarbital anesthesia by inhibiting liver microsomal enzymes (Adams and Dixit, 1970). Use of chloramphenicol with other drugs probably should be restricted, because such use can inhibit the microsomal enzyme system; it may also prolong the action of some drugs and make them

injurious. In fact, the administering of combinations of antibiotics often is contraindicated (Jawetz, 1975). For instance, in the treatment of enteric disease in laboratory animals, a serious problem has been the emergence of antibiotic-resistant strains of bacteria, which complicates effective therapy (Lindsey *et al.*, 1971; Fox *et al.*, 1973). Antibiotics have also altered the pharmacological and biological activities of drugs in several experiments in neurology and neuromuscular responses (Goodman *et al.*, 1974; Phillis, 1974). Small (1968) has documented the adverse effects of antibiotic therapy in guinea pigs and hamsters. Other variables influencing the microbial flora of laboratory animals are antibiotics and sulfonamides, which may contaminate animal feeds, especially when the feed comes from companies that process medicated farm-animal foods. In addition, the cage boards used to collect animal feces and urine, which sometimes become impregnated with antibiotics, may promote selection of resistant enteric bacteria. Two neuroleptics, chlorpromazine and thioridazine, can induce phospholipidosis in humans and animals and cause cytological alterations reminiscent of inherited lipid-storage diseases in humans (Lullman *et al.*, 1975).

These examples are but a few of the readily apparent adverse interactions among drugs and other compounds, microbial flora, or host tissue. The long-term side effects of drug treatments (including the induction of cancer) are much more difficult to pinpoint. Nitrofurans, antibacterial compounds that have been applied in veterinary medicine, have produced tumors in several species of laboratory animals (Cohen *et al.*, 1973, 1975; Croft and Bryan, 1973). Synthetic estrogens, particularly diethylstilbestrol (DES), have been cited as carcinogenic to humans and animals (Burch and Byrd, 1971; Leonard and Diczfalusy, 1974; Edmondson *et al.*, 1976). Chloramphenicol, along with other bone marrow-depressing drugs, has been implicated as a cause of leukemia in people, but not for laboratory animals (Fraumeni, 1967, 1969). Phenytoin (diphenylhydantoin)--an anticonvulsant used to treat seizures in animals and humans--is also under suspicion as a carcinogen (Kruger and Harris, 1972). Tertiary amines--which include oxytetracycline and chlorpromazine (one an antibiotic; the other a tranquilizer)--are a subject of investigation, because they can form, in the presence of nitrites and sufficiently acidic media, the potent carcinogens, nitrosamines (Lijinsky, 1974; Wogan, 1975). This reaction may be activated by dietary nitrites in an acidic stomach or by nitrites in the saliva (Wogan *et al.*, 1975). The use of chlorpromazine or other phenothiazines on test animals should be considered as hazardous for another reason: These compounds stimulate prolactin release in females, which may create the risk of mammary tumors (Turkington, 1972). Iron dextran, a commonly administered drug for correcting iron-deficiency anemia, causes tumors in animals and is associated with sarcomas at injection sites in humans (Robinson *et al.*, 1960; MacKinnon and Bancewics, 1973). Phenobarbital and griseofulvin cause hepatic tumors in treated animals (Hoover and Fraumeni, 1975; Jones and Butler, 1975). Phenobarbital also has interesting properties as a cocarcinogen in rat liver (Weisburger *et al.*, 1975). The antituberculosis agent, isoniazid, has been implicated in the etiology of bladder tumors in humans (Hammond *et al.*, 1967). This finding has particular significance for the management of nonhuman primate populations: Several commercial organizations, including importers of primates, have indicated that the drug is regularly administered to rhesus monkeys for tuberculosis control (Schmidt, 1970; Gibson *et al.*, 1971).

From this relatively small list of agents employed in the treatment of clinical ailments in laboratory animals, one can surmise that other widely used drugs probably cause chronic deleterious effects; thus, the administration of drugs to animals should be minimal, and, if done, its effects should be considered in the final analysis of the test data.

EXPERIMENTAL DOSING OF ANIMALS WITH CHEMICALS

Although most human cancers are of unknown etiology, strong epidemiological and experimental evidence exists for a positive correlation between certain industrial chemicals and cancer. Subsequent investigations have confirmed the carcinogenic potential of such chemicals in a variety of animal species. Numerous arguments have been presented, suggesting that most human cancers have compelling environmental factors in their inception and development (Higginson, 1972). Because of concern for the safety of persons working with 15 of the recognized chemical carcinogens, the Department of Labor's Occupational Safety and Health Administration (OSHA) adopted legislation regarding the use of these particular compounds. In this symposium, Vredevoe has reviewed the legal and moral responsibilities of institutions, investigators, and the technicians working with these and other toxic compounds. Safety guidelines for research involving chemical carcinogens also have been published by the National Cancer Institute. Many of these recommendations apply directly to the management of the animals under experimentation (National Cancer Institute, 1975).

Testing procedures in which the investigator uses laboratory animals to determine if a chemical compound is carcinogenic, mutagenic, or teratogenic have become common practices. Although testing for carcinogenicity or other toxicities of a compound may appear straightforward, many factors, several of which were cited earlier, influence the result of such studies. The route by which the compound is administered, the frequency and dosage of each administration, the duration of the experiment, and the species, strain, and sex of the animal will all affect the manner in which the animals are managed and housed, as well as the safety precautions instituted for each study.

Investigators often choose to administer a test compound via the animal's mouth when it is

a substance likely to be swallowed by humans either intentionally or accidentally (e.g., an unwanted contaminant in food or water). Oral dosing can be accomplished by intubation or by addition of the chemical to the water or food. Gavage is the preferable method, because it is safer (see Figure 3) and because it enables a calculated dose to be administered directly to the animal. Furthermore, the animal can be restrained or sedated, thereby minimizing accidental exposure to the person conducting the test. The risk of exposure can further be limited by performing the exercise in a prescribed biological safety cabinet and by housing the animals in laminar-flow cabinets or appropriate isolators until the risk of exposure to the test material or the metabolites in the urine, feces, and bedding has diminished sufficiently. It is important to know the chemical's clearance rate, which depends on the route of exposure, from the blood, urine, feces, and target organ. The intubation method may be preferable for long-term dosing of a compound if the study is dependent on doses at prescribed intervals (as is common in pharmaceutical research) rather then on daily, low-level ingestion of a compound.

Many studies test a chemical's potency by adding it to the animal's food and water; this method presents inherent risks to the animal technician, however, because aerosols of particulate matter and surface contamination are generated in the working environment. Sansone *et al.* (1977) introduced sodium fluorescein into a rodent diet at a dose of 3,000 ppm; this diet was fed to rats housed in solid-bottom, polycarbonate cages with filter tops. The test diet was fed for 8 days to 188 of 704 rats housed 3 to a cage in one animal room, situated in a double-corridor (clean and dirty) animal facility. The tracer material, fluorescein, was detectable by a spectrophotofluorimeter at a concentration of 0.5 ng/ml of water, and more than 95 percent of the particles were respirable. Although the data should be interpreted with caution, results indicated that both the operations of the animal technicians and the activities of the animals produced and spread contamination, which not only exposed people within the work area, but also those outside the controlled environment. The test material also caused cross-contamination among animals dosed at different levels and housed in the same room; moreover, the control animals were affected, too. The bedding from five cages, which had housed animals whose diet contained fluorescein (390-770 µg of fluorescein per gram of food), was compared with bedding from cages of undosed animals. Although the contamination of bedding from the animals receiving the fluorescein diet did differ markedly from that of bedding from animals that had not received fluorescein, the results showed that the control animals were also contaminated (0.04-0.3 µg fluorescein per gram of food).

Thus, if at all possible, not only dosed and control animals, but also animals receiving different doses, should be housed apart. Small, cubicle-shaped systems with separate air-handling systems (Lang and Harrell, 1969; Poiley, 1974) or laminar reverse-flow cage systems equipped to exhaust air would afford protection against such cross-contamination (Beall *et al.*, 1971), as illustrated in Figure 4. The use of portable and

FIGURE 3 Oral dosing of a manually restrained rat with an intubating tube.

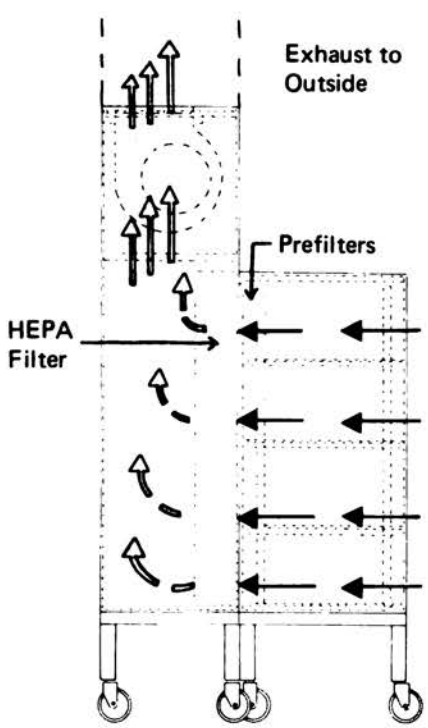

FIGURE 4 Negative pressure, laminar-flow animal housing unit. Air is filtered through a prefilter and a (HEPA) filter.

disposable bedding units with safety cabinets (shown in Figure 5) that blow a negative airflow through the canopy (at 30 m/min) or centralized vacuum systems would reduce an animal technician's exposure to a chemical during the cage-changing process, which can involve handling contaminated bedding (Baldwin *et al.*, 1976).

The concentrations of contaminant in the room--in both the clean and the dirty corridors--indicated that areas with people and unrelated animal cages can become contaminated (Sansone *et al.*, 1977). This finding is illustrated in Figure 6. In general, the greatest contamination occurred on days corresponding to peak human activity in the animal room. The animal room was dry-swept regularly, according to the general animal-care procedures; this activity is not recommended, however, in laboratories testing carcinogens in animals (National Cancer Institute, 1975). In Sansone *et al.*'s study, protective clothing was usually found to be contaminated, as were the technicians' hands above the cuffs of the gloves, their exposed facial areas, the cotton pledgets placed in nostrils, and the respirator filters. Amounts of fluorescein recovered from different areas of the animal rooms and workers clothing and equipment are listed in Tables 6 and 7.

Weihe (1975) employed the techniques of particle counts and phase mapping with microbial organisms to demonstrate that the diurnal, physiological traits of laboratory animals (Cloudsley-Thompson, 1961; Siegel, 1961) can aid in managing animals dosed with hazardous substances in the feed or water. This study measured concentrations of microorganisms and particles during the light and dark periods in animal rooms housing rats,

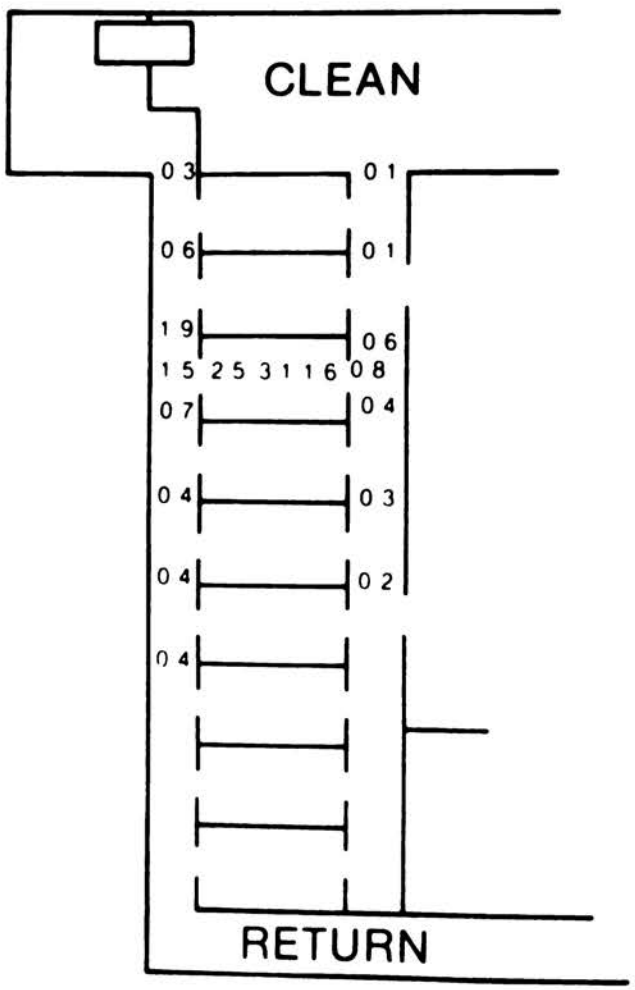

FIGURE 6 Grams of fluorescein recovered from floor samples. Amounts are means for days 5-11 of experiment.

FIGURE 5 Portable unit for disposing of animal bedding, with negative flow of air through the canopy portion of the cabinet.

rabbits, cats, and monkeys. Correlations between both variables existed and allowed high precision in ascertaining contamination from both sources. Because of the rat's nocturnal habits, the emission of particles and microorganisms peaked during the night; the emissions of cats and monkeys, however, increased only during the active feeding period. The activity of rabbits produced equal emissions during the days and nights. The data readily revealed that in the morning the floors in rodent rooms were covered with a dense layer of particles. In view of this evidence, Weihe suggested that:

- Personnel wash the floor in the morning with a wet mop or a wet vacuum before attending to the animals; disturbing the dust will create aerosols in the room.
- Cleaning the floor in the afternoon is not necessary when human activity in the room is minimal during the day.
- Technicians should work in the early morning when the emission count is lowest, thereby reducing the aerosol exposure.

TABLE 6 Fluorescein Recovered from Environmental Samples[a]

Sample Tested	Fluorescein, µg
Scoop	24.9
Cage fronts	
Tracer group	1.1, 1.3, 25.7
Control group	0.1, 0.2, 0.3
Racks	
Tracer group	5.9, 7.9
Control group	1.8, 2.1
Floor swipes	
Return side of room	178.5
Center of room	8.7
Clean side of room	0.2
Return corridor (just out of room)	4.5
Clean corridor (just out of room)	0.2
Clean corridor (where dosed animals' feed tub was placed)	1.1
Dosed feed tub	
Outside	1.0
Inside	1.0

[a]From Sansone *et al.* (1977).

Another important finding was that, after the Monday cage changes, the accumulation of soiled bedding during the week (samples taken at 7:30 p.m. before and 8:30 p.m. after lights were turned off) did not appreciably elevate the microorganism count and actually decreased the particle count. These results contradict those of an earlier study, in which microorganism emissions were the highest on the Mondays before the cage changing (Teelmann and Weihe, 1974).

Weihe (1975) also studied the influence of rat bedding on the number of particle emissions; he compared solid-bottom cages containing "dust-free" sawdust to wire-bottom cages suspended 5 cm above filter paper sheets for collecting feces, food, and urine. The actual values of particle and microorganism emissions did not significantly differ between the two types of bedding. The emissions seemed to stem from the animal and its feces, food, and urine.

TABLE 7 Fluorescein Recovered from Persons' Clothing and Equipment After Feeding of Tracer Diet

Tested Area	Fluorescein, µg
Gloves	12.8
Hands	4.5
Cuffs	63.6
Exposed facial areas	0.5
Body of respirator	1.7
Respirator filters	1.6
Nose plug	0.1
Personal sampler filter	0.3
Shoes (above shoe covers)	6.7
Jumpsuit	78.2
Hat	0.1
Socks	1.1
Shoe covers	22.0

[a]From Sansone *et al.* (1977).

In assessing toxicological risks, one must recognize that the conventional rodent has a much more active microbial flora than do humans. The flora may alter a substance's virulence, appreciably enhancing or destroying it before it can be absorbed and acted upon by the target organ (Northfield and McColl, 1968; Hanna *et al.*, 1973). Moreover, when a human ingests a toxic substance, the metabolites or residues that pass with the feces are permanently eliminated; rodents and rabbits, however, recycle 10-80 percent of fecal residues (depending on diet) because they are coprophagous. The toxic substances being recycled result in increased exposure to the animals and to the humans handling the contaminated bedding.

Using the same fluorescein tracer (Sansone *et al.*, 1977) and incorporating some of the concepts of the emission study (Weihe, 1975), we have undertaken a study to ascertain if semisynthetic diets prepared in the form of gelled casein would reduce the amount and extent of contamination (E. Sansone and J. G. Fox, unpublished data). We compared the contamination among rats housed singly in solid-bottom cages with filter tops to that among rats housed in suspended, stainless steel, wire-bottom cages with paper placed in drop pans to collect wastes. The physical setting was also altered, with the animal rooms situated in a conventional, one-corridor animal-housing system (Sansone and Fox, 1977).

The intravenous or intraperitoneal injection of hazardous substances is difficult to repeat consistently over long periods, and injection itself does not usually duplicate probable conditions of exposure. For strong carcinogens like nitrosamines, however, clearance rates and carcinogenesis can be studied by this route of exposure if, after a limited number of doses, the animals can be maintained so as to avoid contamination of equipment and personnel. For example, the potent carcinogen, dimethylnitrosamine (DEN), rapidly leaves the bloodstream after intraperitoneal or intravenous inoculation and thereby allows the housing of dosed animals with little risk (Rogers *et al.*, 1975). The speed with which DEN is eliminated is documented in Table 8. The clearance of another carcinogen, *N*-methylnitrosourea, occurs within 15 minutes of intravenous injection; evacuation takes 2-3 hours after intragastric administration (McCalla *et al.*, 1968; Swann and Magee, 1968).

The subcutaneous and intramuscular routes, like the intravenous and intraperitoneal ones, allow an accurate dose to be adminstered and decreases exposure to personnel. Administered by these routes, the compound circulates in the body, circumventing those metabolic changes that may occur in the gastrointestinal tract. The interpretation of pathological data is complicated,

TABLE 8 Blood Content of Diethylnitrosamine (DEN) After Intraperitoneal Injection[a]

Time After DEN Injection, min	DEN in blood, μg/ml ± S.E.[b]
4	36.1 ± 2.0
20	19.8 ± 2.6
40	13.9 ± 3.0
60	11.2 ± 3.0
120	3.1 ± 1.5
210	None detectable[c]

[a] From Rogers *et al.* (1975).
[b] Rats received 25 mg DEN/kg of body weight; 4-5 rats were studied at each time period.
[c] Under the experimental conditions, 0.05 μg/ml would have been easily detectable.

however, by local tissue irritation or infection that can erupt after repeated injections of the carcinogen; some have argued that the physical characteristics of the injected substance, such as osmolality or active surface properties, may be responsible for tumor formation (Grasso, 1970).

Hazardous substances have been directly applied to the skin in studies of carcinogens, particularly the polycyclic hydrocarbons (Saffiotti and Shubik, 1963). One advantage of this method is that the required dose tends to be very small and the production of tumors fairly rapid. However, the absorbed dose cannot be measured accurately and, occasionally, the animal will ingest the chemical by licking the exposed area. Darlow *et al.* (1969) explored the environmental hazards produced by experimental applications of carcinogens to the skin. After applying spores of *Bacillus globigii* to a clipped skin area on the dorsums of 30 mice, they noted that the spores remained on the skin and surrounding area for 16 days. Changing the bedding, sweeping the floor, and reclipping the hair of the mice elevated the airborne spore counts during the 16 days after treatment. Although microbial spores do differ from carcinogens in some respects, the fact that airborne particles can remain in the environment dictates that appropriate safety precautions be taken when carcinogens are applied to a test animal's skin.

Studies that test the inhalation of a substance can simulate the effects of natural exposure to compounds believed to increase the likelihood of lung tumors among industrial workers. Inhalation chambers, which deliver known concentrations of the substance to the air, require specialized design and operation. In addition, they are costly to construct and maintain (Hanna *et al.*, 1970; Nettesheim *et al.*, 1970). Individual cages equipped for inhalation studies are also available; they have separate inlet and exhaust filters on racks with exhaust air manifolds (Jemski and Phillips, 1965; Cook, 1968; Chatigny and Clinger, 1969). Other methods include nasal installation of liquids in droplet form or direct tracheal application with an intubating needle, presented in Figure 7. The latter technique has been successfully performed with the carcinogen benzo[*a*]-pyrene for production of respiratory tumors in

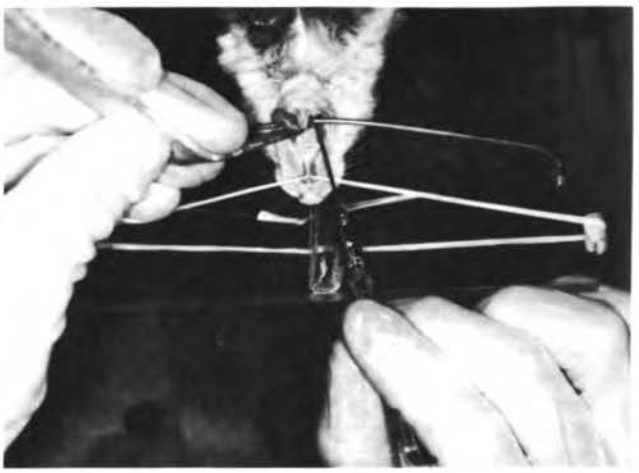

FIGURE 7 Elastic band and fixed heavy wire used to suspend anesthetized hamster also holds the animal's mouth open, thereby easing the insertion of the needle into the trachea. The aluminum speculum is used to depress the tongue so arytenoid cartilages can be readily seen.

the hamster (Smith *et al.*, 1975). This procedure depends, however, on the physical and chemical characteristics of the compound. Intercurrent infection and irritation of mucous membranes, cilia disruption, and altered pulmonary function must be considered; many chemicals elicit a fatal pneumonitis if applied directly in the trachea. It is also important with inhalation techniques to know the fate of the administered carcinogen. One study demonstrated that an appreciable portion of diethylnitrosamine administered to rats was exhaled into the air unchanged (Heath, 1962).

The risks to workers performing these different methods of drug administration are always present, as the various studies have illustrated. Protective clothing and appropriate respirators are recommended when the work environment is likely to be contaminated by aerosols. Inhalation of an unrecognized microbial aerosol has been shown to be the most frequent cause of laboratory-caused infections in humans (Wedum, 1974). Wearing a respirator on the face is not a perfect protection, especially for a bearded person (Hyatt, 1976). Several investigators have reviewed the types of protective face masks or respirators that should be worn during hazardous experiments (Greene and Vesley, 1962; Dineen, 1971; "Data Sheet," 1976). Since one can assume that protective clothing will become contaminated during a carcinogenesis study, instituting disposable gowns, boots, and head coverings should seriously be considered to prevent exposure to personnel.

Protection of personnel should begin by installing primary barriers where the hazard originates. Proper animal caging and biological safety cabinets are protective equipment for minimizing risks. Using this equipment is a routine part of microbial research, and their institution in toxicology studies is certainly indicated (Darlow, 1969;

Sansone and Slein, 1976; Gerone, 1978). Many companies produce laminar-flow cabinets suitable for dosing procedures and housing contaminated animals. These barriers do not, however, provide the safety afforded by metal or plastic isolation systems with accessible glove ports. If dosing with toxic substances will be carried out over long periods, animals should be housed in solid-bottom cages with filter tops. Other systems would be laminar-flow housing units with negative airflow (which exhausts the air to the outside) or gas-tight, Class III cabinet systems with separate exhaust systems. Secondary barriers incorporated into building designs are described by Barkley in this symposium.

Adequate protection of humans depends on valid detection methods to monitor the work area and personnel for contamination. Several researchers have developed sampling methods and analytical means to detect accidental exposure to a number of the carcinogens used in experiments (Haddon *et al.*, 1971; Linch *et al.*, 1971; Garner, 1975; Environmental Protection Agency, 1976; Issenberg and Sornson, 1976). Bioanalyses of urine, blood, and fecal samples are also helpful in ascertaining accidental intake of toxic substances by personnel (Gleason *et al.*, 1969; Christensen and Zeng, 1975).

In establishing safety and research procedures for dealing with hazardous test chemicals in animal studies, a laboratory supervisor should review several key factors influencing the degree of risk:

- the size, species, sex, and number of animals and their behavioral traits (diurnal patterns, activity, aggressiveness, etc.);
- the concentration of the chemical and the route and frequency of its application;
- the chemical's biological activity, physical characteristics (solubility, volatility, specific gravity, particular size), and absorbability through intact skin or even through rubber gloves (Banthorpe and Lamont, 1967; Idson, 1971);
- the volatility of the vehicle;
- the physical makeup of the room (wall surfaces, ventilation, temperature, means of access, and availability of biological safety cabinets) (Yamauchi *et al.*, 1967);
- the type of animal caging, protective filters, and bedding (which should be dust-free);
- the methods of animal-care practices for changes of bedding, frequency of changes, and floor cleaning;
- the methods available for detection, analysis, and deactivation of the compound;
- the type of disposal and cage washer;
- the methods for cleaning or disposing of protective clothing, masks, and equipment; and
- the medical surveillance of personnel,

TABLE 9 Proposed Classes of Chemical Carcinogens for Which National Cancer Institute Safety Standards Will Be Available[a]

I. Aliphatic compounds
  Halogen derivatives
  Nitrogen derivatives
    Hydrazine
    Azoxy
    Diazo
    Nitroso-amines
    Nitroso-amides
  Oxygen derivatives
  Phosphorus derivatives
  Sulfur derivatives
  Carboxy derivatives
    Lactones

II. Cyclic compounds
  Nitrogen derivatives
    Aziridines
  Oxygen derivatives
    Epoxides

III. Aromatic compounds
  Halogen derivatives
  Nitrogen derivatives
    Amino compounds
    Uncondensed rings
    Condensed rings
    Bridged rings
      Methane
      Ethylene
      Azo

IV. Polycyclic aromatic compounds

V. Heteroaromatic compounds
  Nitrogen ring
    N-nitroso
    N-oxide
  Oxygen ring

VI. Structural analogs
  Mustards (*N*-, *O*-, and *S*-)

[a]Listing of chemical carcinogens (OSHA carcinogens are underlined): hydrazine, 1-methylhydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, procarbazine, chloroform, carbon tetrachloride, vinyl chloride, ethylene dibromide, 1,2-dibromo-3-chloropropane, ethionine, urethane, 3-methylcholanthrene, benz[*a*]anthracene, 7-bromomethylbenz[*a*]anthracene, 7,12-dimethylbenz[*a*]anthracene, benzo[*a*]pyrene, polychlorinated biphenyls, β-propiolactone, 1,3-propane sultone, methyl methanesulfonate, ethyl methanesulfonate, bromoethyl methanesulfonate, *N*-(4-[5-nitro-2-furyl]-2-thiazolyl)formamide, *bis* (chloromethyl) ether, chloromethyl methyl ether, diepoxy butane, *p*-dioxane, 4-nitroquinoline-1-oxide, diazomethane, ethylenimine, dimethylethylenimine, propylenimine, *N*-nitrosodimethylamine, *N*-nitrosodiethylamine, *N*-nitrosodipropylamine, *N*-nitrosodibutylamine, *N*-nitrosopiperidine, 1,4-dinitrosopiperazine, *N*-nitroso-*N*-methylurea, *N*-nitroso-*N*-ethylurea, *N*-nitroso-*N*-methylurethane, *N*-nitroso-*N*-ethylurethane, *N*-methyl-*N*'nitro-*N*-nitrosoguanidine, *m*-toluenediamine, 4,4'-methylene *bis*(2-chloroaniline) (MOCA), 4-aminobiphenyl, 4-nitrobiphenyl, benzidine, 3,3'-dichlorobenzidine, 3,3'-dimethoxybenzidine (*o*-dianisidine), 3,3'-dimethylbenzidine, (*o*-tolidene), 1-naphthylamine, 2-naphthylamine, *N*-2-fluorenylacetamide (2-AAF), *N*-acetoxy-2-fluorenylacetamide, *p*-dimethylaminoazobenzene, *o*-aminoazotoluene, 3'-methyl-4-amino-azobenzene, chlorambucil, uracil mustard, aflatoxins, cycasin.

including bioanalysis of blood and waste specimens for evidence of contamination.

These factors help determine the risk category under which a compound should be placed.

In addition, the National Cancer Institute's Laboratory Chemical Carcinogen Safety Standards Subcommittee has identified 63 carcinogens that will serve as the basis for the preparation of 18 monographs on carcinogen safety (Steinman, 1976). These carcinogens, which include the 15 regulated by OSHA, will be classified into groups based on chemical structure and reactivity; Table 9 presents these substances as they were initially grouped. Each monograph will specify the safety data for all members of a particular class.

Another way to assess risk is to designate each compound's ability to produce tumors. Those of highest risk are the chemicals known to produce tumors in humans (Clayson, 1962; Miller, 1970). Of decreasing hazard are those chemicals suspected of being carcinogenic to humans, those shown to be carcinogenic to non-human primates, those producing tumors in two or more animals species, and, finally, those carcinogenic in one animal model. Some of the well-defined human carcinogens include aromatic amines and related compounds, asbestos, and certain nickel- and chromium-containing compounds (Kriek, 1974). The aflatoxins and dialkylnitrosamines belong to classes of carcinogens that are present in specific human environments, and, because they are among the most potent carcinogens known today, these chemicals are highly suspect human carcinogens (Wogan, 1968; Scalan, 1975; Phillips *et al.*, 1976). Although a few carcinogens do not require metabolic activation to express carcinogenic activity, most do undergo such activation to become chemically reactive ingredients, a phenomenon summarized in Table 10. Each compound must be evaluated as to whether its active metabolites are excreted in the feces or urine, as is the case with *N*-2-acetylaminofluorene, whose *N*-hydroxy derivative is an even more active and versatile carcinogen than the parent amide (Cramer *et al.*, 1960).

Additional classes of carcinogens, many of which occur in the environment, are still being discovered; current tests cannot predict the potential dangers of a newly recognized carcinogen or a synthesized one belonging to a recognized class (Maugh, 1974). Techniques have been developed, however, to express the biological activities of certain carcinogens as functions of nonbiological factors, thereby enabling the formation of equations for statistical analysis; such equations can aid in predicting the potency of new compounds (Hansch and Dunn, 1972; Hansch and Clayton, 1973; Wishnok and Archer, 1976).

Carcinogenesis in laboratory animals, and probably in humans, is a multistep process that transforms normal tissue to malignant cells. Many endogenous modifiers can influence this process, such as dependence of reactivity of metabolizing enzymes on species, strain, sex, and endocrinological status of the animals

TABLE 10 Chemical Carcinogens Grouped by Requirement for Metabolic Activation[a]

I. Substances not requiring metabolic activation
- A. Biological alkylating agents
  1. *S*-mustards
  2. *N*-mustards
  3. Epoxides
  4. Aziridines
  5. Alkyl-alkane sulfonates
  6. Strained ring lactones
  7. Nitrosoamides
- B. Inorganic chemicals
  1. Certain metals and metalloids
  2. Asbestos
- C. Radiochemicals

II. Substances requiring metabolic activation
- A. Polycyclic hydrocarbons
  1. Carbocyclic compounds
  2. Heterocyclic analogs
- B. Aromatic amines
  1. Carbocyclic compounds
  2. Heterocyclic amino- and nitro-compounds
  3. Aminoazo- compounds
- C. Nitrosoamines
- D. Hydrazines
  1. Hydrazine
  2. Alkyl hydrazines
  3. Alkyl azo- compounds
  4. Alkyl azoxy- compounds
- E. Hormones and related substances
  1. Estrogens
  2. Goitrogens
  3. Androgens
- F. Miscellaneous
  1. Urethane
  2. Carbon tetrachloride
  3. DDT
  4. Dieldrin

[a]Adapted from David B. Clayson, unpublished.

tested. Exogenous factors, such as diet or certain agents (i.e., carcinogens) in the environment, might affect or induce enzymes required for metabolic activation. Studies that involve simultaneous administration of two or more compounds or the administration of one with an enhancing compound to the test animal may well increase the potency and potential hazard of handling those compounds.

Finally, and most importantly, in assessing carcinogenic or toxicological activity of a compound in laboratory animals, one must also determine the risk to persons working in the animal area. Because irritation and infection

act as cofactors in toxic reactions to tissues (especially of the respiratory tract), they are important considerations in selecting animal technicians for this type of work. People who smoke, have chronic respiratory ailments, or are subject to immunosuppression should not be allowed to engage in animal studies of carcinogenesis. Moreover, animal technicians working on these studies should receive continuing medical surveillance.

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Discussion

GOLDSTEIN: I am James Goldstein, an architect and engineer. Dr. Gerone, you commented that airborne hazards are the most difficult to control, and therefore you recommend avoiding the release of aerosols. Would you recommend that animal manipulations or operations involving possible release of aerosols be considered in a room separate from the animal-holding room of that group?

GERONE: Without question, any procedure involving use of aerosols should be done in a separate facility.

MORELAND: I am Alvin Moreland from the University of Florida. I have a question for one of the microbiologists regarding the tuberculosis vaccine, bacille Calmette-Guérin (BCG). Animals inoculated with BCG are frequently housed with other animals in open or conventional housing areas. Although I am not so much concerned with pathogenesis, I must consider the possible altered immunologic response in other animals if this agent is transmissible. Is it known if the BCG organism will spread to and infect sentinel animals or nonexperimentally infected animals housed near other animals that *are* infected?

VREDEVOE: In some limited work with rodent systems, we were unable to detect altered immunologic responsiveness in cagemates of animals injected, without scarification, with BCG. However, we were examining a rather crude measure of altered immunological responsiveness, i.e., immunotherapy of transplanted tumors. It would be important to examine more carefully the question of altered immunologic reactivity in noninjected cagemates of animals receiving BCG by various routes and doses.

VAN HOOSIER: I am Dr. Van Hoosier from the University of Washington. I would like to question the recommendation about the ultraviolet (UV) lights in laminar airflow hoods and the use of UV lights in general. In my experience, UV lights have been ineffective in controlling infection because of the rapid decrease in intensity with distance and the blunting effect of thin films of dust that collect on them. I would like to ask Dr. Gerone if he has evidence indicating that UV lights are really effective.

GERONE: You have mentioned two of the pitfalls of using UV lights. The intensity does diminish rapidly, and, unless these bulbs are kept properly dusted with a wet cloth, their effectiveness will be lost. Some studies have demonstrated that UV lights do appreciably reduce cage-to-cage transmission of infection. Based on these studies and because UV lights are inexpensive and easy to use, I would recommend using them wherever possible.

COUSINS: I am Oswald Cousins, veterinarian at Lederle Laboratories. I have a question specifically directed to Dr. Gerone. How would you deal with personnel whom you are recruiting into a primate colony in which tuberculosis will be investigated? That is, how do you deal with an individual who is known to be positive for tuberculosis and with a known negative who converts to positive?

GERONE: First of all, we skin-test negative personnel every 6 months. There is no point in skin-testing positive people. Positive people are X-rayed every 6 months. If a person converts from negative to positive, then we institute the treatment that the Center for

Disease Control (CDC) has advised. We recommend isoniazid therapy for 1-2 years.

COUSINS: I have seen that recommendation, but I wonder how widely accepted it is. I suspect our people would prefer to leave us than go on isoniazid therapy in light of current reports on chronic or long-term administration of isoniazid to humans. I have seen two studies, and I would hate to recommend isoniazid to someone. Do you have any suggestions?

GERONE: Well, we would think very seriously about giving isoniazid to anyone over 45, for instance. I do not know what the other contraindications are, but the decision is in the hands of the physicians. We would go by their recommendation. I received the same information from the VA hospital in New Orleans and from Dr. Couch at Baylor University, which they had both gotten from CDC. They would put an individual who converted from a negative skin-test to a positive one on isoniazid for 1-2 years.

COUSINS: Could we recommend to the medical department anything other than isoniazid therapy?

GERONE: X-rays, sputum cultures, and other diagnostic tests may be appropriate. Fortunately, we have not had that situation.

ORTHOEFER: I am John Orthoefer, U.S. Environmental Protection Agency. Dr. Vredevoe, you said that a laboratory worker's pregnancy should not exclude her from working with hazardous agents, but rather more effort should be directed toward containment of the hazard. I agree with that approach. However, Dr. Newberne indicated that the possibility of pregnancy in technicians should be considered when developing an overall safety practice. He also presented data showing higher rates of miscarriage and fetal wastage in pregnant operating-room workers, presumably stemming from exposure to anesthetic agents. My concern is if a congenital abnormality should be manifested in a technician's infant, then would the burden of proof that the hazardous agent was not the cause of the abnormal birth be the responsibility of the principal investigator? Demonstrating this could be a tremendous task, particularly because of the many compounds being tested in the laboratory and the possibility of a break in safety and containment procedures.

VREDEVOE: That laboratory technicians may perceive development of diseases in themselves to be the result of a laboratory-acquired infection will be a factor in many situations. If a person handling new types of animal leukemias later on develops leukemia, or even a different type of neoplasm, he or she may infer that the disease developed as a result of laboratory infection. In the future, ways may be available for detecting whether or not the etiologic agent of the human neoplasm came from the laboratory, but right now such proofs are very difficult to establish. Thus proving or disproving that a disease or abnormal birth was the result of laboratory exposure to hazardous agents would be difficult in any situation in which the potential agent is of unknown pathogenicity for humans.

I think that women must participate in deciding if they will work in a hazardous environment, and they will have to realize that the way that they perform is going to be critical. To exclude them from this decision may result in negative consequences to them in their career choices and options for experimental designs in their research. As you indicated, I favor minimizing laboratory hazards so as to make such decisions unnecessary in the future.

GREENSTEIN: I am Ed Greenstein from the National Institutes of Health. I have two questions for Dr. Vredevoe. One is, how do you protect women who do not yet know they are pregnant? The other question is about the use of committees to control biohazards. Often politics is the overriding factor--thus a great deal is to be desired in the committee control of biohazards where human health is concerned.

VREDEVOE: I realize that by the time a woman perceives that she is pregnant, she is very likely to be well into the first trimester, which is a period of high risk. That is one of the reasons I speak of improving the safety of the workplace.

Now, to look at the committee structure, I think you have a valid criticism. You have to examine the decision-making process in committees and the sorts of advice that the committee receives in making those decisions. We seem to be in a time when the natural response to many problems is to form a committee to look into it.

MYERS: I am Dr. Myers from the Jackson Laboratories. My question is somewhat along the same line. What measures are being taken by NIH to inform researchers about biohazards?

FOX: I will ask Dr. Emmett Barkley of the National Cancer Institute (NCI) to reply to that.

BARKLEY: Having come from a Senate committee hearing on Laboratory Safety yesterday, I can say that you will see a tremendous increase in the amount of support for safety that will be coming from all federal agencies. The safety of the laboratory worker and possibilities of endangering the community by laboratory activities will be receiving much attention.

As far as specific programs that are now under way, the NCI and environmental safety divisions within the NIH have initiated training programs in two areas. One deals with the safe handling of oncogenic viruses and the other, recently developed, concentrates on the safe handling of recombinant DNA molecules.

The NCI has also contracted with Illinois Institute of Technology Research in Chicago to create a laboratory training program for the safe handling of chemical carcinogens. These are demonstrations taught at various locations around the country, and they are open and tuition-free to any laboratory worker or safety professional who wishes to attend. The Institute also is supporting an

applied safety program at the Frederick Cancer Research Center, where much information will be developed on assessing hazards of current techniques used in biomedical experiments. This information will be presented through published literature and a series of annual symposia on safety in research.

Several manuals on laboratory safety are being prepared, and you can expect to see them soon. One very recent one, on recombinant DNA techniques, appeared in the July 1976 *Federal Register* when the NIH guidelines were published. An extensive appendix addressed many areas of laboratory safety.

I would also suggest that you review the excellent literature on laboratory safety coming from Great Britain. To try to bring together guidance on all areas of laboratory safety, the World Health Organization (WHO) is establishing an international program. WHO had an organizational meeting a week ago, and there may be an opportunity to establish international consistency in the areas of risk assessment and recommended safety practices.

The public as well as the laboratory workers have become increasingly aware of the potential for hazard in the conduct of experiments. Their expressions of concern have provided an impetus to accelerating and improving the safety guidance that will be available in the future. So, I think we can look forward to some excellent resources and guidance.

Design Criteria for Animal Facilities

CYRIL B. HENKE

The design requirements for an animal facility are continually being refined as we enhance our knowledge about the environmental factors affecting human and animal health and biological responses in the workplace. The experience gained in applying existing methods and procedures for containing biohazards is an additional basis for a more clear definition of criteria for the design of laboratory animal facilities. The purpose of this paper is to present design criteria and selected design concepts for contamination control and personnel safety in laboratory animal facilities. The criteria and concepts apply to single rooms and to complete facilities for housing animals, ranging from rodents to small primates, and for research involving biological and chemical agents of varying degrees of hazard.

The design criteria are adapted primarily from the National Cancer Institute's (NCI) *Design Criteria for Viral Oncology Research Facilities* (1975a). The Institute's requirements are based on extensive documentation and knowledge of what precautions are necessary for ensuring the minimum acceptable level of environmental control and safety for cancer research. The criteria should be directly applicable to laboratory animal facilities in which a wider range of biological and chemical agents are handled.

DEFINITIONS

New terminology is constantly evolving in the field of biological safety, particularly when discussing facility or safety equipment features that pertain to the level of agent risk. It has become even more confusing with the Department of Health, Education, and Welfare's (DHEW) issuance of safety guidelines for genetic research, in which four "P" levels of containment are classified according to risk (National Institutes of Health, 1976). Barkley (1978) describes the guidelines in his paper in this symposium and relates them to the types of facilities described in this paper. The definitions explained below are presented to avoid any confusion with other guidelines and to clarify the criteria that follow.

Basic Laboratory Animal Facility

One in which animal care and research involving low- and moderate-risk agents can be undertaken effectively and safely. The facility has basic physical features and equipment for minimizing cross-contamination and human exposure to chemical, biological, and microbial agents.

Containment Laboratory Animal Facility

One in which animal care and research involving high-risk agents can be undertaken effectively and safely. The building includes all features of a basic laboratory animal facility with additional architectural and equipment barriers to minimize cross-contamination and prevent the release of any potentially hazardous agents into the laboratory environment or the surrounding community.

Level of Risk

The degree of hazard associated with an agent and/or an operation. The risk levels defined in the National Sanitation Foundation's (1976) standard for Class II biohazard cabinetry are rela-

tively precise and applicable to the various agents used in animal facilities. The risk levels are quoted as follows:

> *Low Risk:* Risk level of agent and/or operation of minimal effect on personnel, other animals, or plants under ordinary conditions of use. This is restricted to all etiological agents designated Class I as specified by the U.S. Department of Health, Education, and Welfare, Center for Disease Control. (National Sanitation Foundation, 1976, p. 2.)
>
> *Moderate Risk:* Risk level of agent and/or operations that require special conditions for control or containment because (a) of known pathogenicity to personnel, other animals, or plants; (b) concentration; and (c) genetic alteration, synergistic effect with other material. This includes all etiological agents in Class II or Class III as specified by the U.S. Department of Health, Education, and Welfare, Center for Disease Control; and oncogenic viruses specified as moderate risk by the National Cancer Institute. (National Sanitation Foundation, 1976, p. 3.)

For our purposes the moderate-risk category will also include suspect chemical carcinogens and chemicals designated as carcinogens by the U.S. Department of Labor (DOL)(1974) when used in dilute concentrations excluded from regulation by the carcinogen standards.

> *High Risk:* Risk level of agents and/or operations that require additional control measures beyond those for moderate risk. These are agents or operations with various dangerous combinations of the following characteristics: (a) low infective doses for personnel, other animals, or plants; (b) high mortality; (c) potential for spread outside the laboratory; (d) concentration; (e) release of microbial aerosols; and (f) genetic alteration or genetic recombination that significantly increases potential pathogenicity or spread. This includes all etiological agents in Classes IV and V as specified by the U.S. Department of Health, Education, and Welfare, Center for Disease Control; and oncogenic viruses classed as high risk by the National Cancer Institute. (National Sanitation Foundation, 1976, p. 3.)

For our purposes, the high-risk category will also include chemicals designated by the DOL as carcinogens when used in mixtures above specified concentrations. Further information on the classification of etiologic agents and oncogenic viruses can be obtained from the *Classification of Etiologic Agents on the Basis of Hazard* (U.S. Public Health Service, 1972) and *Safety Standards for Research Involving Oncogenic Viruses* (National Cancer Institute, 1974b).

Primary Barriers

Enclosures within a room for containing and preventing the release of chemical, biological, or radiological contaminants into the room environment. Usually refers to ventilated hoods such as laboratory hoods or biological safety cabinets

Class I Biological Safety Cabinet A partial enclosure or hood primarily for personnel protection. Protection is provided by inflow of room air through a fixed-size work opening; contaminants are captured in the air stream and removed to site or device where they can be collected, inactivated, or otherwise disposed of safely. Suitable for use with low- and moderate-risk agents.

Class II Biological Safety Cabinet A partial enclosure or hood for personnel or product protection. Personnel protection is provided by an inflow of room air at the work opening; product protection is simultaneously provided by recirculating contaminant-free air through the work space. Suitable for use with all low- and moderate-risk biological agents (NSF, 1976); selectively useful for chemical and tracer-level concentrations of radioisotopes (NCI, 1975b; NIH, 1976).

Class III Biological Safety Cabinet A total enclosure primarily for personnel protection; occasionally used to provide a controlled, clean environment. Protection is given by a physical barrier that is gas-leak tight; work is performed through attached rubber gloves. Suitable for high-risk agents.

Secondary Barriers

Components of a facility that prevent the airborne migration of contaminants among rooms, other functional areas, or to the exterior environment. Components include walls, doors, air locks, pass boxes, air filters, and pass-through sterilizers.

DESIGN OBJECTIVES

Good design for laboratory animal facility embraces two parallel objectives, functional convenience and environmental control. Functional convenience directly affects the conditions under which laboratory or animal-care personnel must work. It is recognized that laboratory and animal-care workers ultimately determine the quality and safety of the facility environment by how well they select and perform their duties. The facility, therefore, must be designed to enhance worker ability to perform animal-care and research activities effectively.

Environmental control can minimize animal or human exposure to harmful agents or conditions, especially those that are related to the air environment. Epizootics, cross-infection, contami-

TABLE 1 Facility Type According to Risk Level

Level of Risk	Facility Type	Comment
Low	Basic	Work conducted on open bench top; animals in open cages.
Moderate	Basic	Agent/procedures confined to Class I and Class II biological safety cabinets; animals confined to filter-top or ventilated cages.
High	Containment	Facility isolated by additional secondary barriers. Agent, procedure, or animals confined to appropriate primary containment devices. Class III containment usually provided.

nation of research materials, and laboratory-acquired diseases can be greatly reduced or eliminated. Animal health, human comfort, and the validity of research data can be improved by local control of such variables as air temperature and humidity and the concentrations of nuisance air contaminants such as dusts and odors. Designs for environmental control tend to be restrictive, i.e., the idea is to isolate and/or confine materials or operations to smaller spaces where they can more readily be controlled. These aims are not always compatible with functional convenience, and therefore compromises must be made to achieve a plan in which both safety and efficiency are acceptable.

Fortunately, many facility features for environmental control and safety have already been designed and tested. Certain features are considered essential for any basic laboratory animal facility testing low- and/or moderate-risk agents. It has been found that functional convenience, environmental control, and safety can be integrated to the advantage of the operating program. When the risk associated with an agent or a procedure is high, additional primary and secondary barriers must be added to the basic facility to make certain that the agents cannot be released to the room or the outdoor environment. The safety features of a containment facility obviously reduce operating convenience, but the inconvenience is small compared to the increase in safety. The general relationship between facility type and level of risk is shown in Table 1.

ARCHITECTURAL CRITERIA

The types of spaces normally provided for animal care include animal rooms, food preparation and storage, cage and equipment washing and storage, waste collection and disposal, quarantine, shipping and receiving, medical treatment, and administrative areas. When research is a part of the program, space is often set aside for animal surgery, experimental treatment, observation, monitoring, and analysis. It is these spaces that must be arranged for functional convenience, with consideration of the following environmental control and safety criteria:

- *Traffic flow patterns:* Flow patterns for personnel, animals, and materials must minimize the proximity of healthy animals, personnel, and clean items to infected animals, animals under experimental treatment, and contaminated items.
- *Isolation of contaminants:* Spaces where contamination sources are known to exist should be separated from areas where materials and animals sensitive to contamination or cross-infection are processed or maintained. The primary sources of contaminants are animal rooms and waste collection and disposal areas.

Space Arrangement--Basic Facility

The arrangement of space to meet the above criteria should include the following considerations:

- Office-type space should be isolated by at least one set of doors from areas where low- or moderate-risk agents are present and animals are handled. Offices should be located to give administrators control of visitor access to work areas. A lunch room or break area should be located outside work areas.
- A change room with a shower is required at the exit of a work area in which persons may be exposed to airborne particles contaminated with chemical carcinogens (National Cancer Institute, 1975b).
- Spaces for feed storage, diet preparation, clean supplies, and equipment should be located as far away as practical from major sources of contamination. Suggested locations are the end of a row of animal rooms, near the entrance for workers, or near the receiving area.
- At least one animal room should have an air lock as an additional barrier to the escape or entry of agents harmful to animals.
- *Room* space (not corridor space) should be provided for the collection, temporary storage, decontamination, or sterilization of solid and liquid wastes.
- Research laboratory rooms where materials such as tissue-cell cultures are handled should not be located adjacent to animal rooms or waste staging areas.

Obviously, not all of the above features can be incorporated fully into a basic facility. For example, the single-corridor concept shown in Figure 1 is a common arrangement in existing and renovated facilities. It results in a counterflow of people, clean items, contaminated wastes, and possibly infected animals. If we assume, however, that all contaminated items are "contained" before they are removed from the rooms, then the potential for cross-contamination is relatively low and the counterflow condition is an acceptable trade-off made in the interests of separating support areas from animal areas. The floor plan of Figure 1 is intended to illustrate the concept and is not necessarily a practical design for an animal-housing program.

The two-corridor concept shown in Figure 2

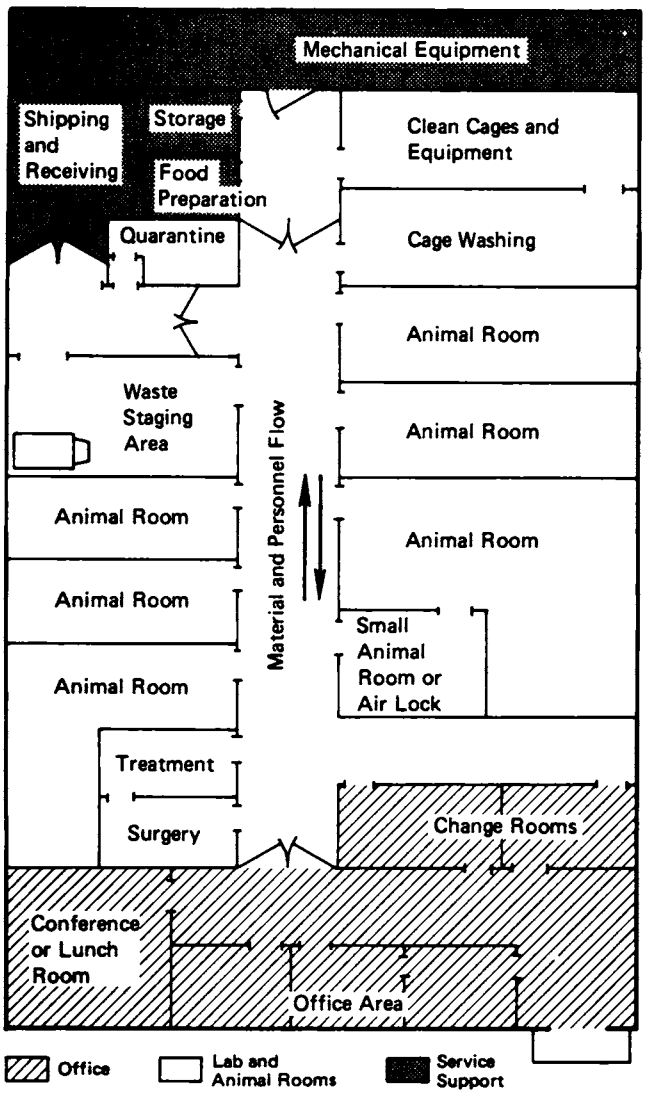


FIGURE 1 A single-corridor design for an animal facility. Diagram courtesy of C. B. Henke.

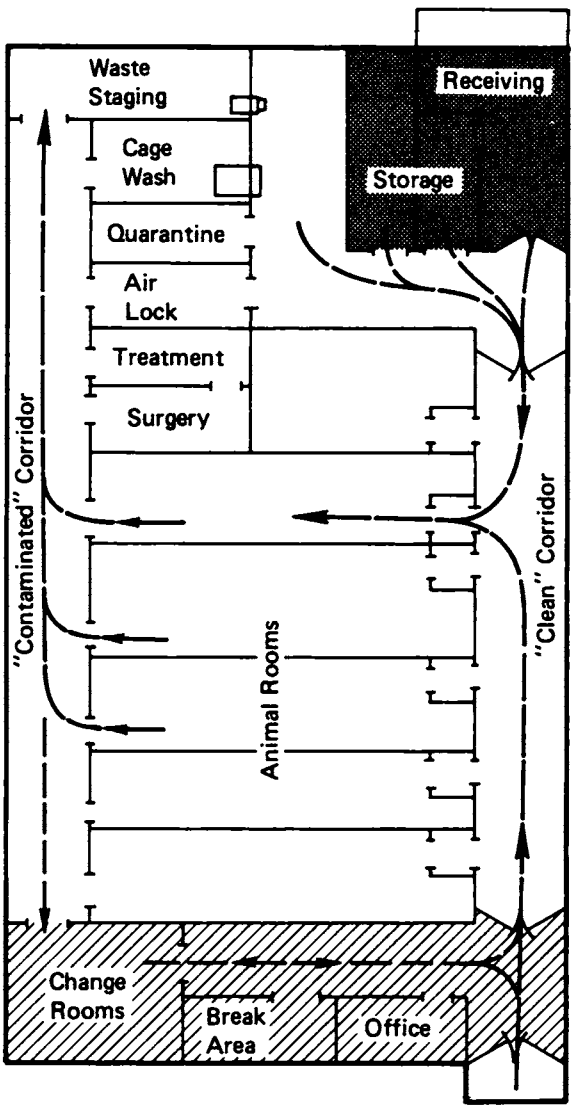


FIGURE 2 A two-corridor design for an animal facility. Diagram courtesy of C. B. Henke.

best accommodates traffic flow and isolation by permitting humans, animals, and material to flow from the areas of least contamination to areas where the contamination is highest (Poiley, 1960; Thorp, 1960; Runkle, 1964; Phillips and Runkle, 1967; Ruys, 1969). This benefit, plus excellent separation of functional areas, usually requires more building space. In larger facilities, two laboratory suites may share a common corridor, which is either contaminated or noncontaminated, depending on the desired arrangement.

Arrangement of Areas for Containment of Hazards

The isolation of laboratory animal spaces can be increased by constructing a separate facility or by adding secondary barriers to a basic facility. Either facility must have the following provisions:

- air locks to permit entry of personnel, equipment, and materials;
- locker room equipped for changing clothes and showering; and
- pass-through sterilizers for removal of laboratory and animal wastes.

Figure 3 illustrates the above features arranged for an efficient flow of people, animals, supplies, and wastes. The loss in operating convenience should be obvious, as is the fact that more space is required to assure that high-risk agents cannot escape from the containment zone.

Construction Methods and Materials for the Basic Facility

The construction of a basic facility with controls for the internal environment can readily be accomplished with standard construction materials and methods. Satisfactory facilities have been built with materials ranging from wood stud

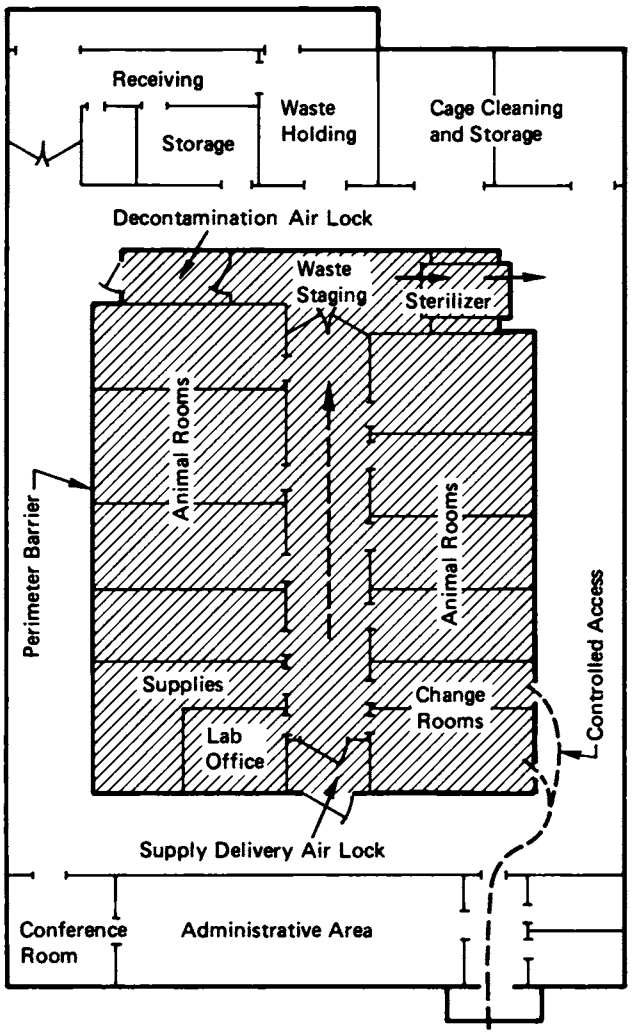


FIGURE 3 A plan for isolation areas in a laboratory animal facility. Diagram courtesy of C. B. Henke.

and drywall to concrete block. The major difference between this type of facility and other facilities is in the quality and degree of completeness of secondary barrier systems. The need to prevent the accumulation of microbial contamination and minimize the airborne migration of contaminants puts greater demands on surface finish quality and the integrity of structural joints and seals. The following basic criteria set forth special construction requirements for environmental control and safety.

Construction Methods The walls, floors, ceiling, and any utility penetration thereof should be constructed and sealed in such a manner as to prevent the passage of insects or rodents and block the migration of airborne contaminants to adjacent spaces.

Construction Materials All surface finishes should be easy to clean and discourage the accumulation of contamination. All finishes should satisfactorily withstand detergent cleaning solutions; floors and walls must withstand exposure to animal wastes and frequent exposure to water.

The selection of construction methods should include the following special considerations for environmental control and safety:

- There should be no visible openings from one work space to another at the wall, ceiling, or floor joints. Openings at utility, electrical, or duct penetrations should not exceed 0.078 cm. If space is to be decontaminated with a gaseous chemical agent such as formaldehyde, tighter seals may be necessary to prevent excessive dilution of gas concentration or gas migration to other areas. For example, air infiltration through utility and ductwork penetrations should not exceed 0.30 m^3/min in a 60-m^3 room if the concentration of formaldehyde gas is to be maintained within 30 percent of the peak concentration for at least 1 hour.
- It is preferred that all walls in animal rooms extend from the finished floor to the undersurface of the floor above. If ceilings are suspended, they must be sealed to meet the previously stated criteria.
- Utility pipes and ductwork should be located so as to minimize horizontal dust-collecting surfaces in work areas. This placement can be accomplished by locating the work space so that the back of the room is against a common utilities corridor or by providing a utilities space on the floor above (Runkle and Phillips, 1969).
- Structural features should be included that will permit partial or total conversion to a containment facility. These features should make it possible to install air locks, change rooms, pass-through sterilizers, and other secondary barrier features required for the future containment of high-risk agents.

Surface finishes within animal facilities normally receive severe exposure to equipment and personnel traffic, animal wastes, housekeeping procedures, and cleaning agents. The high-quality surface finishes normally required to give this service will usually withstand any additional stresses that result from contamination-control procedures. It is beyond the scope of this paper to identify specific construction materials, but the following special factors should be considered in specifying the performance of surface finishes for purposes of contamination control:

- All surfaces should be monolithic--i.e., as free from pinholes, cracks, and crevasses as possible. It seems reasonable that any discontinuity in finish material greater than 0.039 cm should not be permitted.
- All finishes should be free from surface roughness, which would tend to hold contamination and inhibit cleaning. Care must be taken, however, to avoid the selection of floor materials that present a slipping hazard when wet.
- All floor and wall finishes in spaces that must be routinely cleaned and/or decontaminated should be capable of withstanding exposure to solutions containing decontamination chemicals

in any of the following concentrations: 1 percent quaternary ammonia, 1 percent phenol, 0.5 percent chlorine, and 2 percent iodaphor.

- All work surfaces such as laboratory benches and biological safety cabinets should be resistant to the following chemical solutions: 4 percent hydrochloric acid, 4 percent sodium hydroxide, 1 percent quaternary ammonium compounds, 5 percent formaldehyde, 0.5 percent chlorine, 2 percent iodaphor, 5 percent phenol, and 70 percent ethyl alcohol (National Sanitation Foundation, 1976).
- All surface finishes and equipment components should be resistant to 0.8 percent formaldehyde gas in air.

Construction Methods and Materials for a Containment Facility

The same methods and materials used to build a basic facility can be used to construct a containment facility. The difference between the two facilities lies in the degree of tightness of the secondary barrier system. The containment facility must prevent the escape of high-risk agents from the containment area. Its special construction features are:

- The walls on the perimeter of the containment area should isolate this area from all others; isolation barriers should extend from the finished floor to the undersurface of the floor or roof above (National Cancer Institute, 1975a).
- All construction joints and service penetrations in the perimeter barrier must be sealed to minimize air leakage between spaces. The degree of structural tightness necessary to stop leakage in secondary barriers in containment facilities has not been as well defined as it has been for biological safety equipment. In practice, leak tightness ranges from the absence of visible openings to no leaks detectable by a halogen test gas and a halide torch (as used in testing refrigeration systems). To meet the intent of minimizing potential release (or entry of a contaminant) of high-risk agents, seal leak-tightness should approach that achievable by the soap bubble test method in a room at normal, static air pressure. The soap bubble test method, however, is sometimes impractical, occasionally ineffective, and always messy. Thus, a practical and effective method for testing leaks commensurate with acceptable air-leakage specifications does not exist. As an alternative and to be on the safe side, the halogen torch has been successfully used to prove the structural leak-tightness of some government research laboratories.
- Windows in containment laboratory areas should be fixed shut and sealed (National Cancer Institute, 1975a).
- Doors in the periphery of the containment area should be insect- and rodent-proof (National Cancer Institute, 1975a).
- Visual and vocal access from the containment area to other areas is necessary (National Cancer Institute, 1975a). Recommended locations are near sterilizers, pass-boxes, laboratory offices, and supply entry air locks. Flexible film membranes (speaking diaphragms) are available for installation in windows (U.S. Army Biological Laboratories, 1965).
- An emergency exit must be included in the containment area plan. If fire exit doors are necessary, alarm bells should be installed to control unauthorized use. Although not necessarily recommended, knock-out panels have also been installed to provide for emergency egress.

MECHANICAL CRITERIA

General guidelines for the design and operation of animal-care facilities are proposed in the ILAR (1972) *Guide for the Care and Use of Laboratory Animals*. The importance of air conditioning and handling systems is emphasized therein, with specific recommendations for temperature, humidity control, and ventilation. It has been shown, however, that specifying room air conditions may not be sufficient because caged animals may undergo physical stress from increased temperature, humidity, or concentrations of airborne contaminants (Serrano, 1971; Woods and Besch, 1974; Besch, 1975).

A potentially more serious problem than local environmental stress is the presence of airborne contaminants or hazardous agents in the general room environment. It is extremely difficult to control air movement in room-size spaces because most air-supply devices are designed for mixing. Airborne room contamination therefore tends to diffuse and become pervasive unless some basic physical conditions are established to minimize aerosol release, dilute released aerosol concentrations, and confine contaminants to the immediate space.

Every laboratory animal facility, therefore, should have environmental control systems capable of meeting the following basic criteria for the control of airborne contamination.

Ventilation Sufficient air must be supplied to building spaces to prevent buildup of airborne contaminants and enhance temperature and humidity control.

Airflow Patterns The direction of the airflow should miminize the migration of contaminants between functional spaces. It should move towards the area with the highest potential for contamination.

Agent Containment Ventilated safety cabinets or cages must be provided to prevent the release to the room environment of aerosols containing moderate- or high-risk agents. Exhaust air-treatment systems to prevent the release of airborne contaminants to the exterior environment should also be provided.

Ventilation System in a Basic Facility

Air-handling systems should be designed and selected based on the following considerations:

- The *Guide for the Care and Use of Laboratory Animals* (ILAR, 1972) recommends room ventilation rates of 10-15 room air changes per hour. In practice, design recommendations range from 10-20 room air changes per hour, and 15-18 air changes are common (Gorton, 1975; National Cancer Institute, 1975a). Air-change rates should be based on the quantity of air exhausted from the room. Generally, the higher rates are selected more for odor control than the removal of other airborne animal contaminants. In terms of control of particulate contamination, dilution benefits diminish as room air-change rates exceed 10 an hour. Improved housekeeping and more frequent bedding changes can often reduce odors better than increased ventilation.

If individually ventilated cages are used, room ventilation rates can be designed to meet normal thermal requirements of the space. Cage ventilation must be selected according to the heat and moisture load for the particular type and number of animals (Runkle, 1964; ILAR, 1969).

- Laboratory, surgery, and treatment room ventilation rates should be not less than 10 air changes an hour. This rate will provide for a minimum level of air dilution and sufficient airflow for exhausting at least one ventilated safety cabinet or hood (National Cancer Institute, 1975a).
- Air supply to laboratory animal facilities should consist of 100 percent outdoor air all year round. Recirculation of a portion of general room air is technically feasible with HEPA filters (high-efficiency particulate air filters capable of retaining 99.97 percent of a 0.3-μm monodispersed aerosol) and systems for removal of gaseous contamination, but the cost of installing and maintaining the systems and the risk of cross-contamination or human exposure to hazardous agents make the benefit of reduced operating costs a questionable one. For energy conservation, it is better to use a heat-recovery device on the air exhaust. Air exhausted from spaces where chemical carcinogens are present must not be recirculated to any other air supply in the facility (National Cancer Institute, 1975a).
- All animal, laboratory, and service support rooms should be maintained at a negative pressure relative to their respective entry corridors. Clean air should be supplied to entry corridors at a minimum rate of 1.5 m^3/min per room doorway. The level of room negative air pressure is not particularly important and can range from a minimum of a 0.05-cm water column (wc) to a 0.25-cm wc. All rooms within a ventilation zone should be designed to operate at approximately the same negative air pressure. Negative pressures exceeding a 0.25-cm wc are not advisable because the benefits of additional containment are offset by increasingly higher sound levels and interference with door operation.
- In the two-corridor space arrangement illustrated in Figure 2, the most contaminated ("dirty") corridor should be at a negative pressure compared to the rooms. This is a requirement for work areas in which chemical carcinogens are used (National Cancer Institute, 1975b). The general flow of air masses in a "dirty" egress corridor should be from the area of least contamination to the area of highest potential contamination.
- Separate branch supply and exhaust air ducts should be installed in each space to permit proper air balance. Built-in instruments for monitoring airflow quantities and direction are convenient but not essential. A portable air velometer and an inclined manometer can provide the recommended degree of sensitivity for monitoring the air balance. The smoke pencil is an adequate test of airflow direction at corridor doors (National Cancer Institute, 1975a).
- Basic-type animal rooms or laboratories should not be interconnected through the ventilation ductwork with rooms or zones in which high-risk agents, chemical carcinogens (U.S. Department of Labor, 1974), irreplaceable materials, or animals sensitive to contamination are used or maintained. Malfunctions of the air-handling system can result in reversal of normal airflow, drawing air from one room to another through the ductwork. In properly designed and operated systems, airflow reversals are rare; therefore, separate air-handling systems or filtration of the air at the room air supply and exhaust are seldom recommended for conventional or basic facilities. The potential for contamination by this airborne route is orders of magnitude less than by contact contamination.
- The air supply and exhaust to a room, rooms, or zones sometimes must be shut off to permit biological decontamination with a gaseous chemical agent such as formaldehyde (Taylor *et al.*, 1969; Songer and Braymen, undated). If decontamination is performed infrequently (e.g., less than three times a year), then air dampers specifically for sealing off air supply and exhaust are probably unnecessary. Instead, the supply diffuser and the exhaust grill can be temporarily closed off with plastic sheeting and duct tape. A small opening is usually left in the exhaust cover to allow the system to maintain the room under a slight negative pressure. If space decontamination is frequent, shutoff dampers should be provided in ductwork exterior to the room so personnel do not have to enter the space to open dampers. The closed dampers should not permit more than 0.15 m^3/min airflow per 30 m^3 of space (roughly 2-4 percent of the normal volume). A greater airflow is calculated to dilute the required gas concentration by more than 30 percent in 1 hour, the minimum acceptable holding time.
- Ductwork from room spaces or ventilated cabinets/hoods with built-in HEPA filters should meet high-quality standards for conventional ductwork construction. Ductwork and the inlet side of air-filter plenums containing unfiltered cabinet/hood exhaust air must be air-leak tight by the soap bubble leak test at 7.5-cm wc positive pressure. Because this requirement applies to the length of ductwork extending from the cabinet to the filter plenum, it may be more economical to install individual filters and plenums as near as possible to the hood rather than run sealed ductwork long distances to a central exhaust filter plenum. Consideration should be

given to locating filter plenums directly above the rooms in a mechanical equipment space, where they can easily be reached for maintenance.

- Because room air is exhausted by safety cabinet or hood exhaust systems, they are considered part of the total ventilation system and should be designed for continuous operation. If cabinets are under operator control, air by-pass dampers should be installed to keep the room air balance relatively constant. Generally, safety cabinet or hood exhaust blowers should continue operation even if the central supply and exhaust fans fail. There are circumstances, however, when the need to prevent cross-contamination caused by possible airflow reversals exceeds the risk associated with shutdown of a cabinet or of the hood ventilation.
- The safety cabinet exhaust duct should have a static pressure gauge or an airflow indicator so laboratory personnel can check the system's operation.

Ventilation System for a Containment Facility

The ventilation system for a containment facility should have all basic facility features, plus the following extra safeguards:

- The air-handling systems for the containment zone should be separate from other air-handling systems in the facility. They should be designed in a way that maintenance can be performed outside of the containment zone.
- All safety cabinet or hood exhaust systems should be designed to run continuously even in the event of failure of the central supply or central exhaust systems.
- The general (room) exhaust system from the room to the exhaust filter must be air-leak tight at a 7.5-cm wc, as indicated by a soap bubble leak test. The criteria should apply to all ductwork, dampers, access doors, and filter housings.
- Permanently installed instruments should be provided for monitoring the performance of the ventilation system. Pressure gauges should indicate that air is flowing toward the area of highest potential contamination--i.e., negative pressures should exist within the entry corridor, all rooms, and the egress corridor. Automatic alarms should indicate failure of the central supply and any exhaust air-handling systems. In-line airflow monitors are highly recommended for establishing and maintaining air balance.

Treatment of the Supply Air in Basic and Containment Facilities

The basic reason for treating the air supply in an animal facility is to control better the environmental variables under which research involving animals is conducted. Thus, it is recommended that all supply air be passed through filters that are at least 85 percent efficient [dust-spot efficiency test as rated by the American Society of Heating, Refrigerating and Air-Conditioning Engineers (1968)]. This level of filtration will minimize exposure to allergens, dust, and microorganisms that can contaminate experimental materials or infect research animals.

Exhaust Air Treatment in Basic Facilities

There are two categories of exhaust air systems in most laboratory animal facilities: general systems that exhaust room air and local systems that exhaust air from ventilated cages, biological safety cabinets, or hoods. The former requires minimal treatment of air because the concentration of airborne room contaminants must be kept low for human and animal health. The latter is designed to contain and remove moderate- and high-risk agents and requires the highest practicable degree of air treatment. What constitutes "highest practicable degree of air treatment" is obviously debatable. National standards for safe levels of emissions of biological organisms do not exist. Common practice is to specify the efficiency of the contamination reduction component of the air-handling system. The order of magnitude of biological contamination reduction expected for typical system components is summarized in Table 2. Table 2 demonstrates that air-treatment devices can be combined to yield a very high degree of contamination reduction; in addition, the table emphasizes the importance of exhaust stack design in overall system effectiveness. The contamination reduction figures, however, should not be used as design criteria.

The most common methods for treating exhaust air contaminated with particulate material are dilution, filtration, and incineration. Gaseous contaminants are usually treated by dilution and/or incineration. Adsorption or absorption methods are available for special situations.

General Exhaust Dilution is an accepted method for treating room exhaust air in basic laboratory animal facilities. However, two exceptions may require additional air treatment in certain rooms. The DOL's carcinogen standards (U.S. Department of Labor, 1974) require that when certain regulated chemicals are used above specified minimum concentrations, "Exhaust air shall not be discharged to . . . the external environment unless decontaminated" (p. 3,760). Since the designated

TABLE 2 Reduction in Biological Contamination Expected from Typical Air-Treatment Devices

Device	Reduction Expected
Exhaust stack (atmospheric dispersion)	10^2
High-efficiency filters	10^{2a}
Ultra-high efficiency filters (HEPA)	10^{4a}
Incineration	10^{6b}

[a] From Harstad *et al.* (1967).

[b] From Decker *et al.* (1953).

chemicals can be used in "regulated areas" of basic animal facilities, the general exhaust from that space has to be treated to remove detectable quantities of carcinogens. Yet NCI's *Safety Guidelines for Research Involving Chemical Carcinogens* (National Cancer Institute, 1975b) only require dilution by atmospheric dispersion for general exhaust from their intramural laboratories. The NCI recommends its guidelines for any organization involved in research with chemical carcinogens.

A second exception is the recommendation in the *Guide for the Care and Use of Laboratory Animals* (ILAR, 1972) that 95-99 percent efficient filtration (1-5-m particles) be provided for animal room exhaust air in "infectious disease" units. Unfortunately, the "infectious diseases" were not specifically designated, and it is probable that many investigators would construe that some agents in the moderate-risk category are the infectious disease agents referred to in the recommendation. In that case, the scientific investigator should decide if filtration is needed to protect the exterior environment.

In the *Classification of Etiological Agents on the Basis of Hazard* (U.S. Public Health Service, 1972), the treatment of exhaust air is not cited as a facility requirement for working with Class III agents. It states only that air should be decontaminated adequately through high-efficiency filters before it is recirculated. The NCI, however, recommends only atmospheric dispersion as a method for treating general exhaust from rooms in which research involving moderate-risk oncogenic viruses is conducted (National Cancer Institute, 1975a).

Roughing filters may be necessary at the exhaust grill in rooms in which animals that shed excessive amounts of body surface materials are maintained in open cages. The purpose of the filters is to prevent the accumulation of dirt within the air-handling system.

Local Exhaust Local treatment for exhaust air that can be contaminated with moderate-risk agents should be designed to reduce airborne contamination by a factor of *at least* 10^6. This level of reduction can be achieved for particulate contamination through the use of HEPA filters and atmospheric dispersion. For chemical carcinogens, the NCI recommends that local exhaust air be treated "so that the concentration of any chemical carcinogen or combination of chemical carcinogens in the final effluent which is discharged outdoors shall not exceed 1 ppb (part per billion)" (National Cancer Institute, 1975b, p. 22). Although stringent, this standard for National Institutes of Health (NIH) laboratories is more practical than the DOL carcinogen standard, which essentially precludes exhausting *any* of the regulated chemicals. To meet NCI criteria with particulate contamination, double HEPA filtration is recommended along with atmospheric dispersion. Since HEPA filters are incorporated in most biological safety cabinets, a second HEPA can be incorporated in the exhaust system. This arrangement, along with atmospheric dispersion, should be capable of reducing exhaust air contamination by a factor of 10^{10}.

Incineration is recommended for the control of gaseous carcinogens. Air incinerators have the disadvantage of total failure when the heat source is lost, and, when particulate contaminants are involved along with gases, air pretreatment with high-efficiency filters (99 percent or better) is highly recommended. In biological research, air incinerators are most effectively applied to airflow quantities of 3 m^3/min or less.

It is important that all air-treatment systems be designed and constructed to permit maintenance and performance testing without any human exposure to collected contaminants. Provisions must be made for air sampling upstream and downstream of the device, sampling liquid absorption media (absorption devices), decontaminating filters, and replacing contaminated filters.

Exhaust Air Treatment in Containment Facilities

Air exhausted from containment facility rooms should be passed through high-efficiency (95 percent on 0.3-μm particles) filters before it undergoes atmospheric dispersion. Local exhaust for open-face ventilated cabinets can be treated the same as in a basic facility, i.e., single HEPA filtration and atmospheric dispersion.

Because Class III biological safety cabinets are used to contain the most hazardous agents and the built-in HEPA filters are known to develop leaks, it is fairly common practice to provide additional ultra-high-efficiency filtration in the local exhaust system to assure the protection of the external environment. Table 3 summarizes general recommendations for treating exhaust air from laboratory animal facilities.

TABLE 3 General Recommendations for Treating Exhaust Air

Type	Treatment
Basic facility	
General exhaust	Atmospheric dispersion (ad)
Local exhaust	HEPA filtration and/or incineration + ad[a]
Containment facility	
General exhaust	95 percent filtration + ad
Local exhaust	
Open-face hoods	HEPA filtration and/or incineration + ad[a]
Class III cabinets	Double HEPA filtration + ad or HEPA filtration + incineration + ad[a]

[a]When chemical carcinogens are present in the laboratory environment, air-treatment systems must be designed to limit emissions to 1 ppb.

Utility Distribution Systems in Basic and Containment Facilities

All laboratory animal facilities should have the following basic features for contamination control and personnel or community safety:

- Potable water service to the building should be divided into two mains: one reserved exclusively for laboratory, animal room, and support service uses; the other solely for human consumption or use. A backflow preventer must be installed in the first separate main to prevent contamination between the industrial water and potable water systems. Drinking fountains should be located in office areas or in corridors outside laboratory rooms. Ice machines should be connected to the laboratory water system and labeled "not for human consumption" (National Cancer Institute, 1975a).
- Compressed gas cylinders providing carbon dioxide, nitrogen, and other gases should be stored outside laboratory or animal rooms. Permanent manifold piping run from the corridor or central supply room can convey gases to service outlets in the laboratory (National Cancer Institute, 1975a).
- If the research program does not have a policy requiring HEPA filters and liquid traps at each point of use, then HEPA filters should be included in all branch lines of a central vacuum system to prevent contamination of main lines, tanks, and pumps. The exhaust air from the vacuum system should be discharged outdoors in such a manner as to avoid being entrained in any of the building's supply air. Separate, individual vacuum pumps are preferred to central vacuum systems because of the problems associated with maintaining a central system if it becomes contaminated. Separate pumps are recommended for procedures involving volatile carcinogens, providing the vacuum pump exhaust air is discharged through a local exhaust system with the proper air-treatment systems (National Cancer Institute, 1975b).

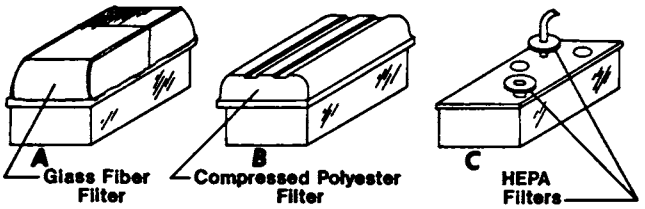
PRIMARY BARRIER EQUIPMENT

Ventilated cages and biological safety cabinets are primary containment devices for preventing the release of potentially hazardous agents to the room environment. They are essential for research involving moderate- and high-risk agents, and their use must be accommodated in new facility design.

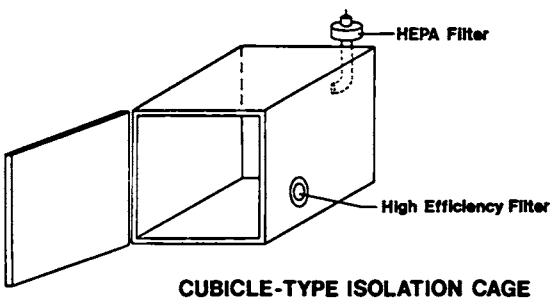
Ventilated Cages

A thorough review of animal caging is beyond the scope of this paper, but because "containment-type" caging influences facility design in general and air-handling system design in particular, a review of the major types follows:

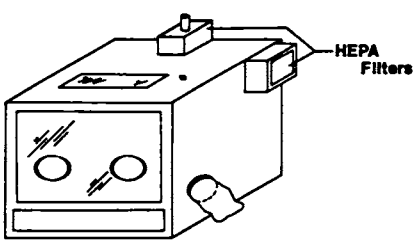
- Filter-top cages A and B shown in Figure 4 are employed most frequently for containing animal-generated aerosols, protecting animals from sudden temperature changes, and reducing the spread of



FILTER-TOP CAGES



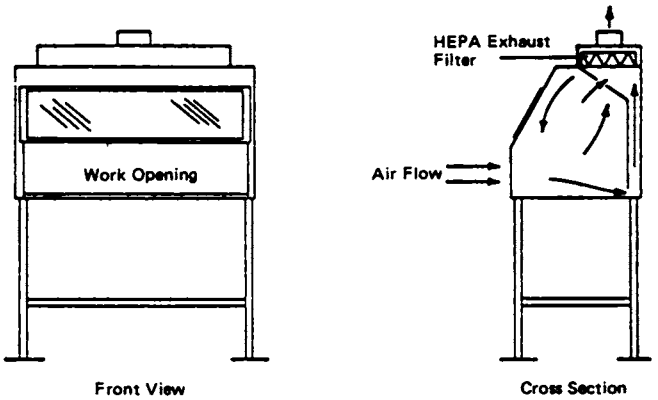
CUBICLE-TYPE ISOLATION CAGE



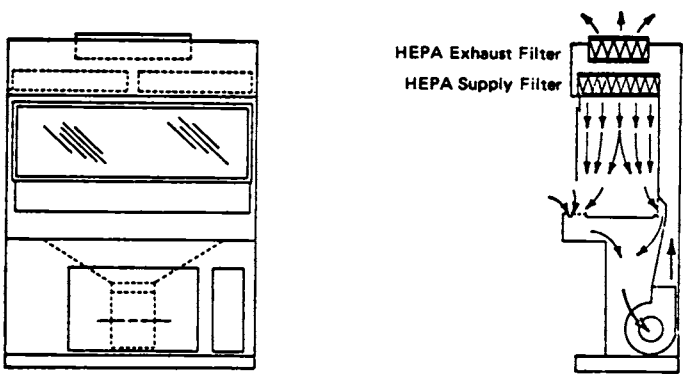
TOTAL CONTAINMENT CAGE

FIGURE 4 Animal cages designed for containing hazards of moderate and high risks. Diagram courtesy of C. B. Henke.

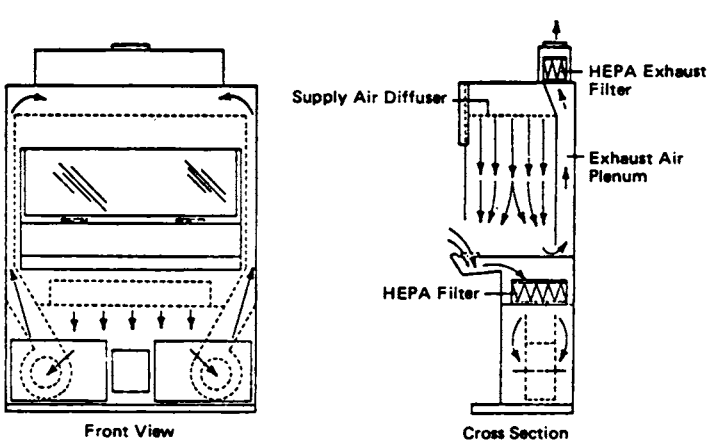
infection among cages. These enclosures for partial containment are recommended by the NCI for animals inoculated with moderate-risk oncogenic viruses (National Cancer Institute, 1974b) or exposed to nonvolatile chemical carcinogens (National Cancer Institute, 1975b). They have a disadvantage, however, in that the filter closures may result in higher temperatures and humidity, elevated ammonia, and carbon dioxide concentrations that can change an animal's biological responses (Serrano, 1971; Woods and Besch, 1974; Besch, 1975). The ventilation system must therefore be designed to distribute air uniformly to the animal room in such a manner as to maintain a healthy environment for all animals, taking into consideration the use of open or enclosed cages (Woods, 1975). Rates of air ventilation in cages should be selected to maintain temperature and humidity recommended for the particular species being studied (Runkle, 1964; ILAR, 1969). When moderate-risk agents are involved, the cage covers should be removed under conditions that will provide additional personnel protection. It is recommended that an open-face, ventilated hood be provided in the animal care area for



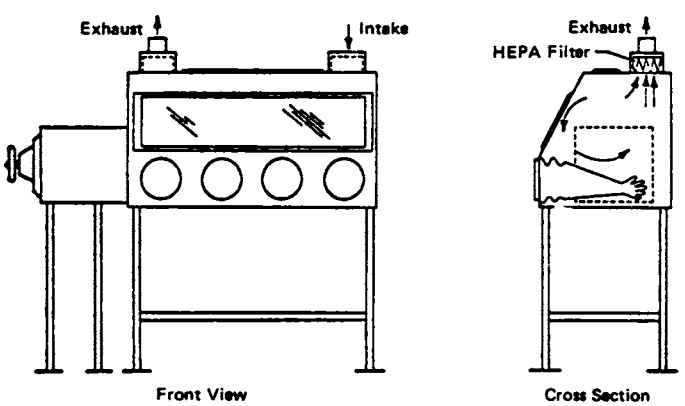
Class I Biological Safety Cabinet



Class II [Type I] Biological Safety Cabinet



Class II [Type II] Biological Safety Cabinet



Class III Biological Safety Cabinet

FIGURE 5 Types of biological safety cabinets. Diagram courtesy of C. B. Henke.

manipulations involving contaminated filter-top cages.

- Filter-top cage C, illustrated in Figure 4, is a forced ventilation cage capable of preventing entry or release of particulate contamination. It is much more effective in containing hazardous substances than cages A or B, without their major disadvantages. Cages are usually manifolded to a single exhaust pipe on a cage rack. An exhaust manifold with multiple flexible hose connections is required in animal rooms in which this type of cage is used; it can be connected to either a local or a general exhaust system. Cage ventilation rates vary with cage size, animal species, and population. An order of magnitude estimate of airflow is 0.03 m^3/min per cage. When connected to a local exhaust-air system with suitable air treatment, this cage can be used in experiments involving volatile carcinogens (National Cancer Institute, 1975b).
- The cubicle-type isolation cage (Horsefall and Bauer, 1940) is a partial containment unit capable of holding one or more standard animal cages. It is effective in preventing cross-contamination among animals infected with different low- or moderate-risk agents in the same room. It provides partial protection for humans, but only when the door of the cubicle is closed. The unit is ventilated by drawing in room air through a high-efficiency filter and exhausting it through a duct located on the rear wall. It is recommended that a HEPA filter be installed in the cage exhaust duct or at the suction end of a manifold exhaust duct for several cages. Air that has been cleaned by a HEPA filter can be removed by a general or a local exhaust system. The volume of airflow required varies with the size of the cubicle and the number, size, and species of animal. An order of magnitude estimate is 0.30 m^3/min for a 0.24-m^3 unit.
- The containment cage in Figure 4 houses animals inoculated with high-risk agents or protects animals from airborne particulates in the environment. If the cage is made halogen gas-leak tight, it is considered a Class III biological safety cabinet. Ventilation air is drawn into and exhausted from the cabinet through HEPA filters that are incorporated into the cage design. Air in containment cages should be exhausted through a local exhaust air system. The exhaust airflow rate is estimated at 0.30-75 m^3/min for a 0.24-m^3 cage, depending on number, size, and species of animal and the presence of other heat-producing devices such as lights.

Biological Safety Cabinets

Biological safety cabinets contain aerosols created during laboratory preparation of substances for experimental use and during animal inoculation, intubation, necropsy, autopsy, and dumping of cage bedding. Three basic types of safety cabinets are illustrated in Figure 5. These cabinets are why laboratory facilities usually have a local exhaust system in addition to a general one. The local exhaust system can accommodate the relatively high air-pressure drop across the HEPA filters incorporated in the cabinets and assure continued operation of selected cabinets in case the general exhaust system fails.

TABLE 4 Exhaust Air Requirements for Biological Safety Cabinets[a]

Cabinet Type	Approximate Exhaust Airflow, m^3/min: Cabinet Length, 1.2 m	Cabinet Length, 1.8 m
Class I	6	9
Class II (Type 1)	7.8	12
Class II (Type 2)	7.5	10.8
Class III	*b*	*b*

[a]From National Cancer Institute (1975a).

[b]20 changes of cabinet air volume per hour or design for actual internal thermal or moisture load.

Standard operating performance criteria have been established for each class of cabinet (National Sanitation Foundation, 1975). Class I and Class II (Type 1) cabinets are required to have an airflow velocity of 22 m/min in the plane of their fixed work openings. This velocity establishes the cabinet exhaust airflow rate, which is summarized for all cabinets in Table 4.

The Class II (Type 1) cabinet has its own internal blowers that permit operation independent of any other air-handling system. Although the air exhausted from the cabinet can be discharged to the room, this procedure is not recommended, because undetected leaks in exhaust filters can result in hazardous agents being released to the room environment. Exhausting cabinet air to the room also prohibits the use of any toxic or odorous vapor or gases. Instead, air should be exhausted through the room (general) exhaust system by means of an exhaust air canopy over the cabinet exhaust filter (National Sanitation Foundation, 1975). The exhaust canopy should not be connected directly to the cabinet, yet it should remove the same quantity of room air when the cabinet is not operating by bypassing the exhaust filter.

The static air pressure required to operate Class I and Class II (Type 2) biological safety cabinets varies according to the design of the cabinet. The HEPA filter is the major resistance to airflow. HEPA filters are normally selected for initial (clean) pressure drops ranging from a 1.5-2.5-cm wc. Because space for filters is limited in Class III cabinets, smaller size filters with an initial pressure drop of 2.25-2.5-cm wc are usually selected. The pressure drop across air intake and exhaust HEPA filters has to be considered in calculating the total pressure drop for Class III cabinets. Class III cabinets are usually operated at a negative pressure of 1.25-1.75-cm wc.

The exhaust system should be designed to accommodate a buildup of twice the original static pressure as the filter becomes loaded with particulate matter. The manufacturer's guide should

be consulted for operating pressures for specific pieces of equipment.

Air-volume dampers should be installed in the exhaust duct (downstream of the HEPA filter) for adjusting the airflow and for sealing off the cabinet while it is being decontaminated with formaldehyde gas (National Cancer Institute, 1974a). The function of the valve is to prevent the migration of formaldehyde gas from the cabinet space and minimize the negative pressure on the temporary plastic cover over Class I and II cabinet work openings. When closed, the damper should therefore present a pressure drop of at least a 5-cm wc. Although they are of excessively heavy construction, commercially available butterfly valves with rubber seals are frequently used for exhaust air dampers. All biological safety cabinets should be performance-tested *at least* once a year (National Cancer Institute, 1974b, 1975b; National Institutes of Health, 1976).

Special Equipment for Contamination Control

The National Cancer Institute, through a contract with the Dow Chemical Company, has for over 10 years sponsored the development of safety equipment to control aerosol hazards in NCI and its contractors' laboratories. Three recently developed cabinets are directed toward the control of aerosols in animal facilities.

The Clean Air Animal Containment System, shown in Figure 6, was developed to provide a means of housing animals infected with moderate-risk agents without extensive renovation of an existing facility. This partial containment device can provide personnel protection, prevent the spread of infections among animals in adjacent cage cubicles, minimize cross-infection within a cubicle, and minimize the animals' exposure to airborne room contamination. Standard cages are maintained in open-face cubicles mounted vertically on opposite sides of the room. Clean air is supplied downward into the aisle between the cubicles, drawn inward past cages, and exhausted at the rear of the cubicles. The air is recirculated through HEPA filters.

Routine experimental and animal-care procedures such as examination, inoculation, bleeding, bedding changes, and feeding can be conducted safely and efficiently at the cubicle's opening directly in front of the cage. The containment capabilities of a prototype unit at the Frederick Cancer Research Center in Frederick, Maryland, were challenged with the introduction of biological aerosols and with animals infected with highly contagious agents. This unit was shown to be effective in preventing human exposure and cross-infection among animals (Barbeito, 1974). An advantage of the system is that animals can be cared for without the restrictive encumbrances typical of containment enclosures. The system cost about $10,000 in 1973.

A second containment system, diagramed in Figure 7, was designed according to the same principles except that air flows horizontally across the cubicles. It was designed for housing primates infected with moderate-risk agents. It cost approximately $25,000 in 1975 and is being used by Meloy Laboratories of Springfield, Virginia.

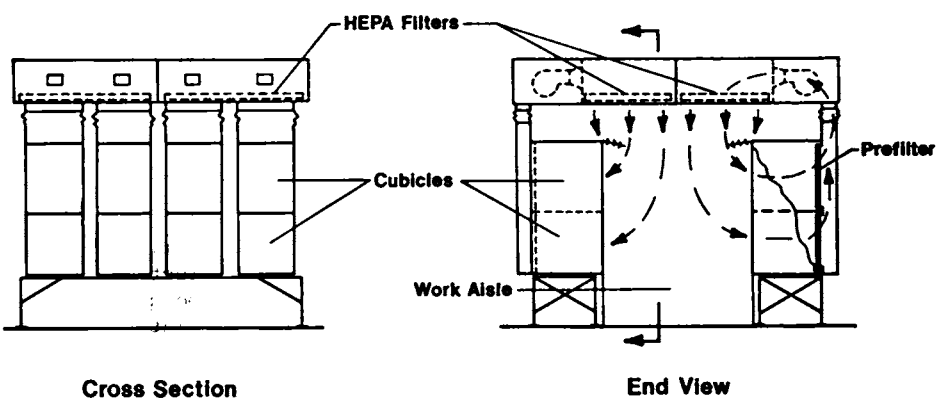


FIGURE 6 A partial containment system in which clean air flows into cubicles. Designed to house various animal species in standard animal cages. Diagram courtesy of C. B. Henke.

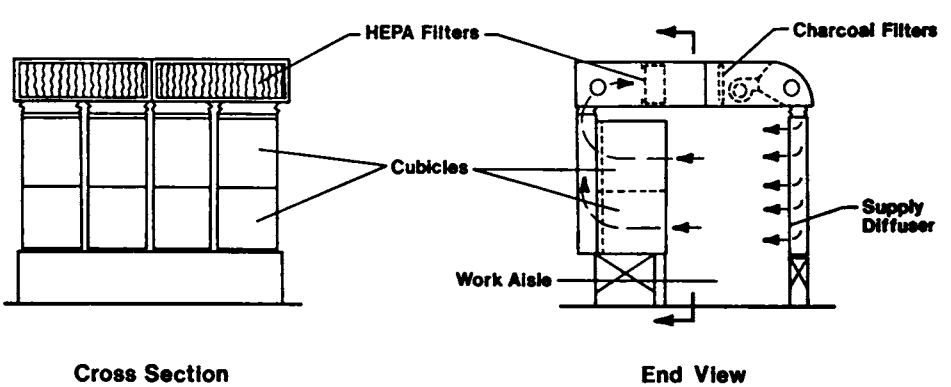


FIGURE 7 A partial containment system in which clean air flows horizontally into the cubicles. Designed to contain primates housed in standard primate cages. Diagram courtesy of C. B. Henke.

Both of these systems are of modular design for assembly within an existing room. They can be disassembled, moved, and reassembled in another location. They require a room height of at least 3.3 m. Both units recirculate about 360 m^3/min of air and add approximately 3,182 watts to the room space, making this method of containment energy-intensive and costly. It is believed, however, that the benefits of safety and contamination control combined with savings in manpower can offset the capital and operating costs for selected research programs.

A mobile cabinet for disposing of bedding, shown in Figure 8, controls the aerosols generated by dumping soiled bedding into waste cans (Baldwin *et al.*, 1976). This equipment for partial containment is of the Class I safety cabinet type, and it permits the dumping of bedding from rodent-size cages into a waste can lined with a plastic bag. The unit is small enough to be moved between cage racks and from room to room. A prototype unit has passed performance tests for biological containment. The cabinet's utility was not fully accepted by animal-room workers in two separate trial runs, because it took up too much space and it required additional effort to operate it. In one, the work surface was too high for the operator's comfort. The cabinet can also be used as a ventilated hood for performing inoculations or transferring animals. Since cabinet air is discharged to the room, it should not be used when chemical carcinogens are part of the research. At present, it is recommended only for those laboratories that do not have a sterilizer available for treating contaminated animal wastes. A prototype unit cost approximately $2,300 in 1974.

A cabinet for performing necropsies on small animals, shown in Figure 9, has been developed to give veterinarians and technicians a more clear, direct view of surgical procedures while simultaneously providing personnel protection and minimizing contamination of animal specimens.

This partial containment unit is similar to a Class II (Type 2) biological safety cabinet except that the view window is sloped vertically for better viewing of the work surface. No air is recirculated within the cabinet. This configuration results in more air turbulence in the work zone, and product protection is estimated to be reduced roughly 10 percent from standard Class II design. The cabinet design has passed biological aerosol challenge tests for personnel protection. The cabinet can be used with moderate-risk agents, low toxicity or flammable vapors or gases, and tracer-level radioisotopes. It is particularly useful for operations involving anesthetic gases. With air velocity of 27 m/min at the work opening, the cabinet requires a local exhaust capacity of 8.4 m^3/min. A prototype model of this cabinet cost approximately $2,000 in 1974.

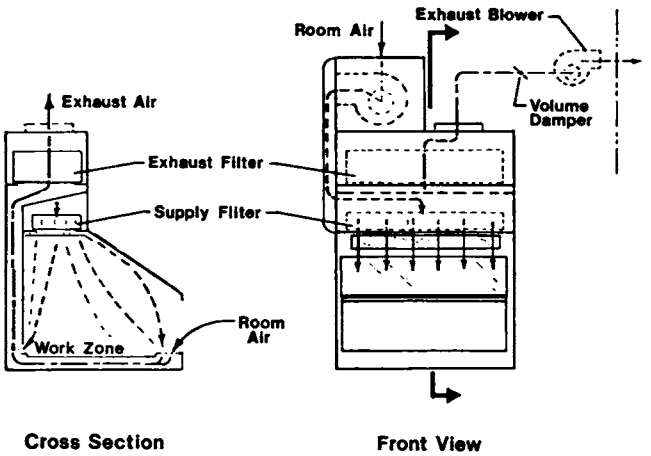


FIGURE 9 A partial containment cabinet for performing necropsies on small animals. Diagram courtesy of C. B. Henke.

Drawings and engineering specifications for the three previously described pieces of safety equipment are available from the NCI's Office of Research Safety, Bethesda, Maryland.

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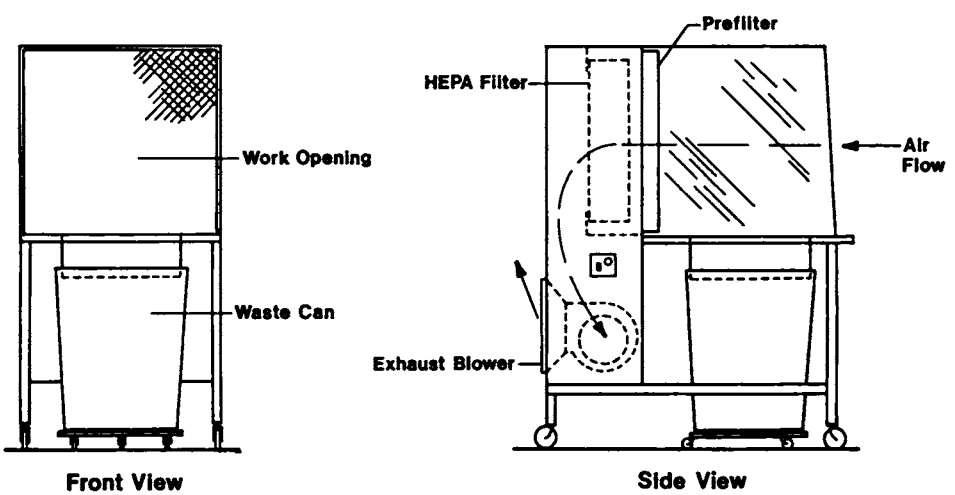

FIGURE 8 A mobile cabinet for disposing of soiled bedding from small animal cages. Diagram courtesy of C. B. Henke.

is based. Dr. W. Emmett Barkley also collaborated with us in that effort. The editorial assistance of Mrs. Pam H. Errico is sincerely appreciated. The special equipment for contamination control mentioned in this paper was developed under contract NOl CP 33243 for the National Cancer Institute.

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Abilities and Limitations of Architectural and Engineering Features in Controlling Biohazards in Animal Facilities

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The existence of biohazards associated with the use of laboratory animals in biomedical research has been well documented. In an analysis of 3,921 laboratory-acquired illnesses (Pike, 1975), the source of infection resulting in 754 illnesses among laboratory personnel was either attributed to or associated with laboratory animals. The infectious process involved with most laboratory-acquired illness, however, is not well understood. Proven accidental exposures account for less than 20 percent of all reported illnesses. Potential exposures by inhalation of undetected aerosols of infectious materials (Sulkin, 1961; Sulkin *et al.*, 1963) and by direct contact with animals and animal wastes (Darlow, 1972) may contribute appreciably to occupational illness among workers in animal facilities.

The control of biohazards in animal facilities depends on the resistance of the laboratory worker, properties of the infectious agent, properties of the laboratory animal, experimental techniques and procedures, methods of primary containment, engineering features of the facility, decontamination and disposal practices, and discipline (Darlow, 1972).

GENERAL REQUIREMENTS FOR THE ANIMAL FACILITY

The animal facility can consist of a single animal room, a complex of animal rooms and appropriate support areas within a laboratory building, or a separate building. The single-room facility is improper for purposes of quarantine or breeding. Although the single-room facility may add some flexibility and convenience to a research program, it does not lend itself to adequate measures for controlling biohazards. Most authorities recommend that laboratory animals be housed in centralized facilities that have the capacity for biohazard control and total animal care (Runkle, 1964a,b; Jonas, 1965; Lang and Harrell, 1969; Runkle and Phillips, 1969; Darlow, 1972).

The principal engineering features of the animal facility that contribute to biohazard control can be divided into three areas: architectural design, ventilation, and environmental protection. Architectural features include the composition of the individual animal room, the organization and arrangement of the animal rooms with respect to support and service areas, and the provisions for and location of architectural barriers such as air locks and changing rooms for personnel. Ventilation features include the manner in which air is introduced into and removed from the facility, direction and rate of airflow, and relative pressure differentials. Equipment for treating contaminated air and liquid and solid wastes is a part of environmental protection.

A typical facility for housing laboratory animals would have a clean access corridor leading to the animal rooms and research laboratories. Entrance to this corridor would be through an architectural barrier such as an air lock or changing room. This barrier would prevent unauthorized persons from entering the clean corridor. The relative air pressure within the clean corridor would be higher than that within the animal rooms and research laboratories. Thus, the air supplied to the clean corridor would flow into the laboratories and animal rooms.

Primary ventilation for the animal rooms would come from a ceiling grill or diffuser, with a rate of 10-20 air changes per hour. Air distribution and control within the animal room

could be aided by a recirculating system employing a mass airflow (McGarrity *et al.*, 1976). The surfaces of the animal room would be prepared to facilitate cleaning, decontamination, and vermin control.

Egress from the animal rooms would be into a second (or dirty) corridor leading to a waste staging area and a changing room. Treatment equipment for sterilizing or decontaminating wastes and dirty cages would be available in the waste staging area. Relative air pressure within the dirty corridor would be lower than the air pressure within the animal rooms.

The exhaust air from the animal facility would be treated by passage through high-efficiency air filters before discharging it to the atmosphere.

ANALYSIS OF ENGINEERING FEATURES

An estimate of the effectiveness of engineering features of animal facilities in the control of biohazards was made by examining records of laboratory-acquired illnesses from which laboratory animals were the probable source of infection. Of particular value to this analysis were occurrences in which no primary safety or facility barriers had been installed as preventive devices. The purpose of the analysis was to determine if the presence of engineering barriers would have prevented any of the laboratory-acquired illnesses.

Review of the literature, summarized in Table 1, yielded 16 accounts of multiple laboratory-acquired illness in which research animals were the likely sources of infection. A total of 365 laboratory illnesses was involved. The causative agent in these illnesses includes 3 Class 2, 7 Class 3, and 2 Class 4 agents, as classified by the Center for Disease Control (U.S. Public Health Service, 1974). These agents caused 41, 208, and 116 illnesses, respectively.

There were no primary safety barriers in these laboratories. The animals were not housed in the typical animal facility described above. No architectural barriers for control of access were available. As a rule, the animals were housed in rooms dispersed throughout the laboratory facility No special facilities or double-door autoclaves for sterilizing solid wastes had been installed. Controlled directional airflow was not provided in any of the facilities, and the exhaust air was not filtered. Descriptions of the ventilation system in these laboratories were, in most cases, not given. It can be assumed, however, that at best the ventilation equaled conventional practices (i.e., 10-15 air changes per hour with single-pass air).

In the 5 accounts summarized in Table 2, the presence of engineering features typically used in the control of biohazards would have reduced the incidence of laboratory-acquired infections. All illnesses associated with the remaining 12 accounts involved persons who had intimate contact with the infected animals and contaminated animal products or with the activity involving the infected animals. Their illnesses could not have been prevented by engineering features of the facility.

ARCHITECTURAL FEATURES AND THE CONTROL OF BIOHAZARDS

Three microepidemics were found where the total number of laboratory-acquired illnesses could have been reduced by effective control of access to contaminated areas. Most notable of these was an outbreak of lymphocytic choriomeningitis (LCM) described by Hinman *et al.* (1975). In this account (A), the source of the infection was Syrian hamsters that had been inoculated with a tumor cell line contaminated with LCM virus. Infected hamsters were housed in one room of a radiation facility and in two rooms of a vivarium located near the radiation facility. Twenty-one illnesses were observed among persons who had no direct contact with

TABLE 1 Microepidemics of Laboratory-Acquired Illnesses Associated with Laboratory Animals

Disease	Probable Source of Infection	Persons Infected	References
Erysipeloid	Dissected horse	13	Gross, 1940
Leptospirosis	Handled infected mice	8	Stoenner and MacLean, 1958
Louping ill virus	Intranasal inoculation of mice	3	Rivers and Schwentker, 1934
Lymphocytic choriomeningitis	Infected hamsters and fomites	10	Baum *et al.*, 1966
Lymphocytic choriomeningitis	Infected hamsters	48	Hinman *et al.*, 1975
Psittacosis	Infected parrots	11	McCoy, 1930
Q fever	Animal dust	15	Hornibrook and Nelson, 1940
Q fever	Infected hamsters	35	Feldman *et al.*, 1950
Rift Valley fever	Handled agent and infected mice	11	Smithburn *et al.*, 1949
Tularemia	Handled and dissected rodents	6	Lake and Francis, 1922
Murine typhus	Intranasal inoculation of mice	6	Loffler and Mooser, 1942
Murine typhus	Intranasal inoculation of mice	12	Van den Ende *et al.*, 1943
Vesicular stomatitis	Handled agent and infected animals	54	Patterson *et al.*, 1958
Viral hemorrhagic fever	Airborne dry rodent urine/feces	113	Kulagin *et al.*, 1962
Yaba-like disease	Handled infected monkeys	5	Hall and McNulty, 1967
Yaba-like disease	Handled infected monkeys	15	Espana, 1970

TABLE 2 Illnesses That Might Have Been Prevented by Engineering Features for Biohazard Control

Account	Total Illnesses	Number of Illnesses Possibly Prevented	Principal Means of Prevention	Reference
A	48	23	Control of access	Hinman *et al.*, 1975
B	11	8	Control of direction of airflow	McCoy, 1930
C	15	4	Control of direction of airflow	Hornibrook and Nelson, 1940
D	35	35	Sterilization of solid wastes	Feldman *et al.*, 1950
E	113	110	Control of direction of airflow	Kulagin *et al.*, 1962

the hamsters, but who had access to the animal room in the radiation facility. These persons generally entered the animal rooms for purposes of visiting and using a copying machine located there. Two illnesses were observed among the vivarium staff who did not handle the infected animals, but who had access to the rooms in which the infected animals were housed. Hinman *et al.* (1975) concluded that this outbreak clearly demonstrated the importance of controlling access to animal quarters.

Although accounts B and C cannot be evaluated as precisely, a common characteristic was the unrestricted movement of personnel throughout the facility and animal areas. In account B, eight persons who had no association with the research project acquired psittacosis. In account C, Hornibrook and Nelson (1940) recorded laboratory-acquired illness among four persons who did not come into intimate contact with animals.

These accounts of laboratory-acquired illnesses reconfirm the fact that persons entering animal areas are at risk. This risk can be eliminated for persons not directly involved in animal experimentation by preventing their access to the animal facility. Architectural features such as air locks and changing rooms can aid in this control.

The value of the architectural features of the animal room and the design layout of the animal facility in preventing laboratory-acquired illness cannot be extrapolated from an analysis of the accounts described above. Attention to separation of animal-care functions in the design of animal facilities can minimize unnecessary contact with infected animals and animal products. The selection of surface treatments can contribute to space decontamination and cleaning and reduce the amount of residual contamination on room surfaces.

VENTILATION FEATURES AND THE CONTROL OF BIOHAZARDS

It is difficult to ascertain the importance of the intraroom ventilation system design or ventilation rates in the control of biohazards. Kethley (1963) has demonstrated the effects of conventional ventilation practices on clearance of aerosols from rooms. He has concluded that no ventilation rate that mixes airborne particles with supply air substantially reduces the inhalation dose that occupants of a room will receive during the initial moments following a burst release of particles. Aerosol wastes in animal facilities are frequently emitted during activities requiring intimate contact with the animal. Examinations, changing bedding, and providing feed are common examples. Figure 1 demonstrates that the airborne particle concentration immediately following an aerosol burst is essentially the same regardless of ventilation rate. The potential exposure for an occupant of a room during the first few seconds following accidental release of particles into the air will, therefore, be the same regardless of ventilation rate. It can be concluded that, with even the high ventilation rates common to mass airflow systems, an occupant will not be protected from exposures during an aerosol burst. The persistence of aerosols from a burst source, however, will be reduced with time, and this reduction *is* dependent on the ventilation rate.

Conventional ventilation systems providing turbulent mixing are virtually ineffective in reducing airborne contamination caused by a continuous source of particles (Chatigny and Clinger, 1969). Figure 2 shows the contamination concentrations established with conventional ventilation practice (e.g., 6-15 air changes per hour) from a continuous source of 10,000 particles per minute.

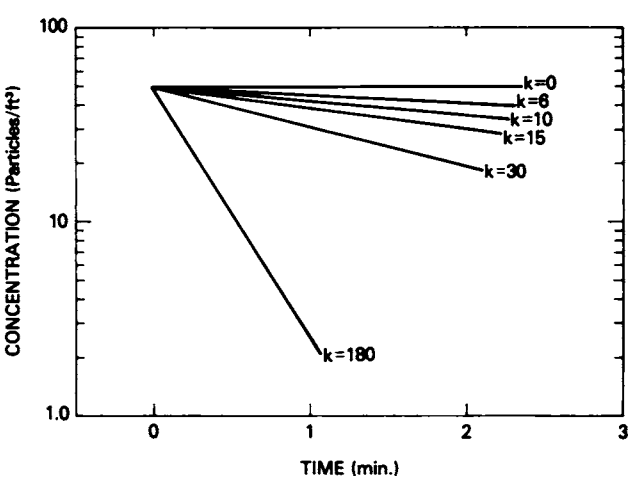

FIGURE 1 Calculated clearance rates of burst aerosol source in a 6 X 3 X 3 m room. The burst source generates 100,000 particles; *k* is ventilation rate in air changes per hour. Figure courtesy of Dr. David West.

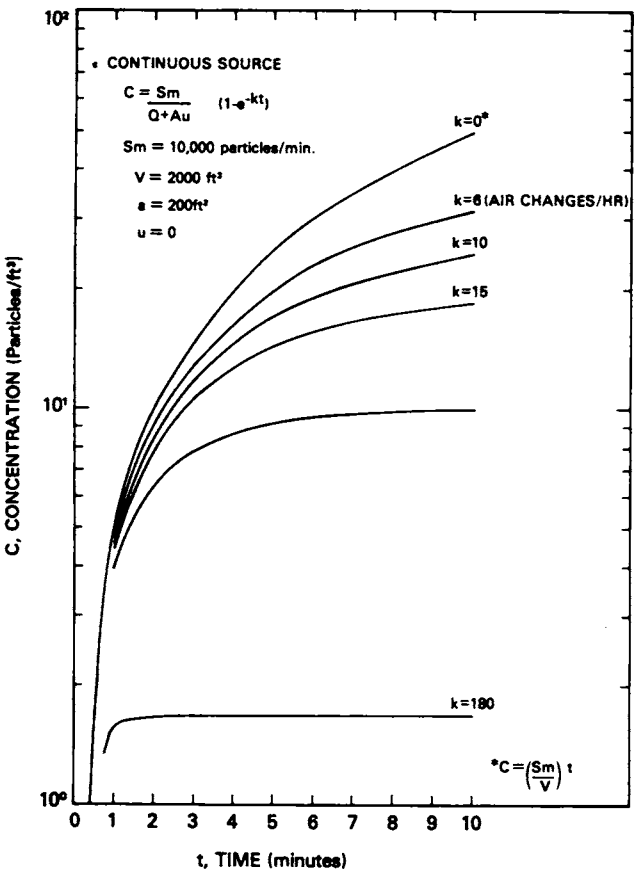

FIGURE 2 Persistence of aerosols from a continuous source in a 6 X 3 X 3 m room. The continuous source equals 10,000 particles per minute. Figure courtesy of Dr. David West.

An appreciable reduction in contamination can be achieved by using a mass airflow system. McGarrity *et al.* (1976) have shown the ability of such systems to lower infection rates among laboratory animals. However, this capability may not be reassuring from the standpoint of human protection since the variation in size of infectious dose for any one agent may vary significantly. Protection of persons in an animal room can, therefore, only be assured by preventing the release of the infectious agent into the room. Caging devices have been developed to screen workers from biohazards and they also provide excellent protection against cross-contamination among laboratory animals (Schneider and Collins, 1966; Kruse and Wedum, 1970).

An examination of accounts B, C, and E indicates that directional airflow--that is the movement of air from areas of lowest potential hazard to areas of highest potential hazard--may play an important role in biohazard control. In these accounts, illnesses occurred among persons who *never* entered the rooms in which the animals were housed. The probable determinant in these accounts was the dissemination of aerosols from the animal rooms throughout the facilities. This is the only factor that can explain the infection of 110 persons in account E. Defective air balance was also shown to have made possible the transmission of hog cholera in a large animal isolation facility of the National Animal Disease Center at Ames, Iowa (Sullivan and Songer, 1966). It must be recognized, however, that directional airflow does not protect the occupants of the animal room; its protective quality benefits those persons who have access to the facility but whose access to the animal quarters is restricted.

ENVIRONMENTAL PROTECTION FEATURES AND THE CONTROL OF BIOHAZARDS

Evidence that the public has been endangered by any research activity involving biohazardous materials is conspicuously lacking. It has not been possible to find reports of illness among persons of the general population who were not associated in some way with a laboratory facility. Only one account (E) of public illness has been documented in which an association existed with laboratory animals. This report involved 35 employees of a meat-rendering plant who were infected with Q fever following exposure to contaminated animals received from a laboratory facility. This incident could not have been prevented, even if environmental protection features had been available. Only attention to standard microbiological practice, which demands sterilization of contaminated wastes, would have prevented the outbreak.

An animal facility should be able to accommodate equipment for sterilizing contaminated liquid and solid wastes. There is no epidemiological basis for filtering general ventilation exhaust air for low and moderate biohazards. Filtration of exhaust air, however, is recommended for animal facilities containing high-risk agents or deliberate aerosol experimentation.

SUMMARY

The control of biohazards associated with animal experimentation involves personnel and operational practices, primary containment devices, and certain facility features. The engineering features of the animal facility have important, but limited, ability to control biohazards. Architectural barriers, ventilation, and environmental protection systems serve to reduce or eliminate the spread of microorganisms that may be accidently released within the animal room. Those features protect those persons who do not enter the animal quarters within the animal facility. Protection from biohazards for humans who do enter animal rooms must be provided by other means of biohazard control.

The importance of architectural and engineering features in the control of biohazards is compared in Table 3. The numerical ratings given in the table are based on an arbitrary scale of 4. On this basis, the more effective devices, such as strict personnel and operational practices, primary containment devices, and discipline in the safe conduct of animal experimentation, would receive a 4. Barriers for access control are the most important of the architectural fea-

TABLE 3 Comparative Importance of Architectural and Engineering Features of Animal Facilities in the Control of Biohazards

Features	Rank
Design	
Room composition	1
Space arrangement	2
Access barriers	3
Ventilation	
Conventional ventilation practices	2
Mass airflow system	2
Ventilation rate	2
Directional airflow	3
Environmental protection	
Exhaust air treatment	1
Liquid effluent treatment	3
Solid waste treatment	3

tures, and thus they receive a 3. General exhaust air treatment is not highly protective in the control of biohazards and therefore receives a 1.

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Training and Surveillance

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My paper will center on the human element in the control of biohazards, including motivation and training of personnel at various levels of the laboratory hierarchy and on the difficulties in maintaining surveillance to ascertain how well the hazard-control program is functioning. Motivating or convincing a senior scientist is a basic and necessary step in formulating an effective program for safety. These motivating factors are related not only to human safety, but also to the equally convincing argument that containment protects experimental procedures against unwanted contaminants. Pike (1976), who documented nearly 4,000 instances of laboratory-acquired infections, has pointed out that many others undoubtedly occur without being reported. Thus the case for personnel safety is amply supported by emerging concerns for ethical practice in scientific research. The degree of experimental contamination and its high cost in lost time, money, and scientific reputation is more controversial. The controversy has been publicized by Nelson-Rees and Flandermeyer (1976), with their claims about widespread HeLa cell contamination of many cell lines, and by Engel and Askanas (1976), who have raised similar questions about avian oncornavirus genus C in chick embryo muscles.

The impetus for establishing strict safety programs in research institutions is great. Recent publicity about the hazard potential of recombinant DNA molecule research is a good example of this trend. The role of the public in sharing the decision-making role in scientific research is being debated and undoubtedly will result in greater public surveillance over the scientific community. This inevitability alone has become an important factor in increasing safety awareness of principal investigators and senior scientists, some of whom have been reluctant to spend money and time on hazard containment.

To buttress the moral and practical reasons for hazard containment, federal regulations are also being enacted to convince the scientist of this need. Government regulations such as the Occupational Safety and Health Administration (OSHA) Act of 1970 are in operation, and it is only a matter of time before a specific OSHA standard for laboratory work will be formulated. Federal funding agencies can also provide an incentive for safety improvements by awarding money for safety equipment specifically requested in contracts or grants. The National Institutes of Health's (NIH) *Guidelines for Research Involving Recombinant DNA Molecules* (1976) specifies the need for compliance with the safety regulations as a condition for NIH funding.

Research institutions themselves will probably apply increasing pressure on scientists to comply with hazard containment. Biohazard committees are being formed at many institutions to see that government regulations are being carried out. These committees should also help to strengthen environmental health and safety capabilities in the institutions. The willingness of federal agencies to provide funding for safety services and equipment to scientists would greatly soften the difficulties of following new regulations. Institutional environmental health and safety departments, which would routinely inspect and certify laboratories, and when necessary decontaminate laboratory hoods and building ventilation systems, collect hazardous wastes, and

provide problem-solving expertise, would obviously be a great asset to the investigator. For the scientist, then, motivation to uphold safety measures takes many forms; it appears to be reaching a new high as pressures and incentives for greater safety awareness are applied.

For technical personnel in the laboratory, safety training and supervision become more important. Ideally each laboratory should have a supervisor who is officially designated as being responsible for safety. The safety program in the laboratory should then include the following elements:

- Rules and regulations. Established policies such as prohibition of mouth pipetting, eating, smoking, and drinking and regulation of laboratory visitors should be instituted.
- Safety action plans. For each experimental procedure, a plan should be developed that takes into account all possible safety or contamination risks and details an effective method of handling each of them. Particularly important are plans for dealing with such emergencies as a spill of a hazardous agent or the escape of an infected animal from a cage.
- Formal and informal training programs. Laboratory safety courses emphasizing biohazards in viral oncology research have been sponsored by the National Cancer Institute (NCI) since 1972. More than 600 laboratory workers have received instruction in these training sessions. One of the program's objectives is that trainees will use the knowledge gained and the reference materials obtained to train others in their laboratories. The NCI has also prepared other training materials, including slides and tape cassettes on various topics concerning laboratory safety. These aids are available on loan to laboratories.

Training aids can also be used to indoctrinate lower-echelon personnel such as animal handlers and glassware washers. These workers will also require closer supervision to ensure that they are following established procedures for dress requirements, hand-washing practices, and safe handling of bedding and other waste materials. Again, clearly defined procedures that take into account all of the potential hazards of the operation are essential to success of the program.

Once laboratory safety procedures have been put into practice, it is important to monitor the program to see if it is working. Such surveillance can take a variety of forms. One of the most basic is physical testing of building and/or cabinet ventilation systems to assure compliance with accepted standards or regulations. Certification of biological safety cabinets is an example of this type of monitoring. Table 1 summarizes the tests necessary to certify a biological safety cabinet. Included are leak tests of the air plenum and of the high-efficiency particulate air (HEPA) filters and velocity measurements at the open face and over the work surface. Even more basic would be a simple test that the operator could perform to check if air is moving in the proper direction. As mentioned, institutional provision of a cabinet certification service would be a major asset to research laboratories.

Microbiological monitoring is another way of determining how well laboratory design and equipment are functioning to minimize exposure of the worker to aerosols. Dimmick *et al.* (1973) have published extensive evaluations of aerosol exposure in laboratories. Their information is useful in determining the degree and type of aerosol containment necessary for given research operations. In general, however, it is not recommended that research laboratories attempt to employ microbiological monitoring as part of their surveillance. The cost of the equipment and the pitfalls in the interpretation of results suggest that such evaluations should be left to specially trained investigators. Vogl and Chatigny (1973) have developed a mathematical nomogram by which knowledge of aerosol generation can be applied to predict the actual exposure a laboratory worker or animal would receive from a particular operation.

Still another effective surveillance technique

TABLE 1 Certification Tests for a Laminar-Flow Biological Safety Cabinet[a]

Test	Equipment Necessary	Desired Result
Plenum leak test	Halogen leak detector	No leaks greater than 8.9×10^{-5}cc/s at 5-cm water gauge pressure
Inflow velocity	Thermoanemometer	Minimum of 22.5 linear m/min variation ±1.5
Downflow velocity	Thermoanemometer	All readings >13.5 linear m/min. Individual readings not to vary more than ±20 percent
Filter leak test	Dioctyl phthalate generator: Light-scattering photometer with probe	Downstream count not to exceed 0.01 percent of the upstream count
Noise level	Decibel (db) meter	Not to exceed 65 db$\underline{A}$[b] (background 55 db$\underline{A}$)

[a] Adapted from NIH, 1974.

[b] $\underline{A}$ scale: The decibel scale that emphasizes the frequencies most applicable to human hearing.

TABLE 2 Immunization Recommendations for Research Laboratories[a]

Vaccine	Indications	Contraindications
Anthrax	All laboratory personnel when agent is present	None
Bacille Calmette-Guerin	*Not* recommended	--
Botulinium toxoid	Workers in direct contact with agent or toxin	Discontinue if severe response is noted
Cholera	Persons working with agent; caretakers of infected animals	Effect in pregnant women is unknown
Diphtheria and tetanus toxoids	All adults should have protection, including booster doses	Febrile illness or history of severe reaction
Eastern equine encephalitis	All workers in laboratory where agent is handled	High sensitivity to egg material
Viral hepatitis immune serum globulin (ISG) (for hepatitis A only)	Handlers of recently imported non-human primates	*Intramuscularly* only
Measles	All susceptible individuals working with agent	Malignancies; febrile illness; immunologic impairment
Plague	Persons working with agent; caretakers of infected animals	None
Poliomyelitis	All workers in laboratory where agent is handled	None
Q fever	Workers or visitors in laboratory where handled; caretakers of infected animals	Persons previously infected or vaccinated with Q fever; egg sensitivity
Rabies	High-risk group--veterinarians, animals handlers, people working with agent, including handlers of glassware, and visitors to laboratory where agent is handled	Allergy to eggs
Rocky Mountain spotted fever	All workers in laboratory where handled; caretakers of infected animals	Egg sensitivity
Rubella	All susceptible individuals who enter lab or animal-care areas where agent is handled	Pregnancy--women of child-bearing ages; altered immune state
Russian Spring-Summer encephalitis (RSSE)	All persons working in or entering laboratory, including handlers of glassware or media	Give only to healthy adults
Smallpox	All persons working in or entering laboratory or building containing the laboratory	Eczema; chronic dermatitis; pregnancy; malignancies; altered immune competency
Tularemia	Workers in laboratory where agent is handled; caretakers of infected animals	Pregnancy; eczema; chronic dermatitis
Typhoid	Workers in laboratory where agent is handled; caretakers of infected animals	Acute illness; chronic diseases; immunosuppressive therapy
Typhus fever (epidemic)	Persons working in or entering laboratory where agent is handled; caretakers of infected animals	Egg sensitivity
Venezuela equine encephalitis	All persons working in or entering laboratory where agent is handled; caretakers of infected animals	Pregnancy
Yellow fever	All persons working in or entering laboratory where agent is handled; caretakers of infected animals	Egg sensitivity; immunosuppressive therapy

[a]Adapted from U.S. Public Health Service, 1974.

is intermittant visual observations of personnel practices. The supervisor should watch new employees for repetitive breaks in safe techniques before they become habitual. It may also be desirable on occasion to have an outsider knowledgeable in safe practices observe the laboratory operation. Even experienced workers may drift unknowingly into an unsafe technique that the supervisor may not recognize because of overfamiliarity with the procedure.

The final element in a good surveillance program is the all-important employee health program. Muchmore (1975) has summarized the elements of such a program for primate facilities, but most of her observations are equally pertinent to other laboratory animal operations. Preemployment physical examinations, including blood serology, are a necessary first step in determining a baseline of employee health. Theoretically, exposure to hazardous agents can subsequently be measured by changes in the baseline data. For overt accidents or acute infections, such data will not be very helpful, except that predisposing conditions that contraindicate employment may be discovered. For long-term exposures related to chronic illness, however, changes are a potential indication of exposure and thus a means for determining if more stringent containment is necessary.

The preemployment examination can also uncover allergic sensitivity to animal dander or other allergens likely to be encountered. Muchmore also suggests that this examination can be used to predict attitudes about safety hazards and animal care, a tool that could aid in ascertaining suitability for employment. In addition to allergic potential, predisposing factors that could contraindicate employment include chronic illnesses that reduce immune competence, continuing use of steroids or other immunosuppressive medications, pregnancy (or likely pregnancy), which should exclude women from work with viral agents, and family history, which may, for example, indicate genetic predisposition to cancer and thus preclude work with potentially carcinogenic agents. Ideally, the physical should be followed up by an equally thorough examination (again, including blood serology) on an annual basis or after return to work from any illness that may have markedly altered the baseline information.

Immunization programs are another important, and sometimes controversial, element in the employee health program. Table 2 summarizes the U.S. Public Health Service's recommendations for immunization practices germane to laboratory animal facilities. Some protective immunizations are recommended generally (such as tetanus and the more controversial rabies) and others only in situations in which the agent is actually being investigated.

SUMMARY

Biohazard containment in laboratory animal facilities is a complex and increasingly important endeavor. Many pressures and incentives are being exerted to motivate laboratory directors toward greater attention to such containment. For workers in these facilities, good supervision, well-organized laboratory safety programs, and formal training courses should be emphasized. The attitudes and awareness of personnel are paramount to safe procedures, even when facility design and equipment are of the highest order. Good personnel practices can also serve to overcome deficiencies in laboratory design and equipment.

Surveillance to ascertain the effectiveness of containment facilities and practices is also important. Physical and microbiological testing, mathematical modeling, and visual observation are among the monitoring techniques available. Finally, employee health programs, including definitive preemployment examinations and regular follow-ups, must be instituted to measure the effectiveness of the preventive safety measures.

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Discussion

CARPENTER: I am Dr. Bob Carpenter from M. D. Anderson Tumor Institute in Houston. Dr. Barkley, if you design a P-3 facility in accordance with the recombinant DNA guidelines, will it be adequate for chemical carcinogen studies?

BARKLEY: A lot depends on what carcinogen you are working with. If you were to assume for a moment that you are working with a particulate system, even a particulate system in suspension, then the P-3 facility would be consistent with the guidelines that the National Cancer Institute has recently issued for experiments involving chemical carcinogens. The next factor is what type of work you are going to do. If you are going to be doing aerosol studies, I think you would have to look at your facility very closely. For most types of bioassay programs involving these materials, however, a P-3 level is sufficient.

SCHNEIDER: I am Henry Schneider from Hahnemann Medical College and Hospital in Philadelphia. Today we have heart about regulations and enforcement. We have also heard about the responsibilities of the institution and senior investigators. A topic of some concern to me is that of liability. We, who are not physicians, are not covered by liability insurance. If we sit on a human studies or institutional research committee, reviewing and passing on research proposals, there is a great concern that, although we assume that we are being covered by the institution, we can be sued, collectively as a committee and/or singly as individuals. We are now seeking some clarification of this issue at several of the institutions in our area. I wonder if anyone has information on this subject, or if any criteria studies have been made for developing the information we need.

FOX: We seem to be fumbling with that. Does anybody have any information on this subject?

WINDLE: I am Doug Windle from Michael Reese. Three months ago, *Business Week* reported a federal case in which a supervisor was personally sued by an employee and was found liable for failing to ensure that the employee was following established regulations.

ORNETH: I am Jim Orneth from Fieldstone Corporation. My compliments to the panel. I would like to address a comment to Dr. Vesley. I am concerned with the use of blenders for tissue-culture work in cases when the technician inserts his or her arm into a biohazards-type hood. I have seen men and women take their hands out and wipe their mouths, rub their eyes and so forth, and then reinsert their hands and continue to work. Also, we place a fair amount of reliance on the monkey mask as protection for personnel. Some crude tests that I have run indicate that they are not terribly efficient at stopping particulates.

VESLEY: Your comment on working in safety cabinets is a very good one. It is another thing that we emphasize in our courses. The safety cabinets afford very good protection against aerosols, but they do not in any way protect against contact contamination. This is part of the awareness and part of the training that must be instilled. You have to be aware that everything within that cabinet can be contaminated by the aerosol, even if you contain it. Therefore, the surfaces remain contaminated, the

gloves will be contaminated, and the technician must act accordingly.

HANSON: I am Jack Hanson from Hahnemann Medical College and Hospital in Philadelphia. I am concerned with the dependence on the high-efficiency particulate air (HEPA) filter. The contaminants are trapped in the HEPA filter itself, and our maintenance people refuse to touch them. Now we are left with the problem of handling a filter that is loaded with contaminants. Nor is any attempt made to prevent the ducts leading to the filter from becoming contaminated. The only solution appears to be to find some method of decontaminating the filters before we change them.

HENKE: There are filter-plenum systems available, which will allow you to remove and bag a contaminated filter and insert a new filter without exposing the personnel. The filter used to be marketed by Barnaby Cheney, but I cannot remember who is handling it now. I also recommend the incorporation of HEPA filters into the safety cabinet. You can then shut off the damper, seal up the cabinet, and decontaminate it with formaldehyde. Your cabinet can then be opened up and the filter replaced. As far as room exhaust filtration goes, we recommend 95 percent filtration on containment room exhaust. The filter should be located as close to the room as possible to avoid installing airtight ducts.

NIMS: I am Dr. Nims from Microbiological Associates. Mr. Henke, you were talking about airborne contaminants in containment rooms. I believe you said that there should be individual controls on the air pressure or an individual air supply to each cubicle.

HENKE: For the purposes of air balancing, each room should have its own individual supply and exhaust duct. You can have standard damper controls within the branch ducts to that room.

NIMS: In multiple suites, if one door is left open the system doesn't work, unless you have an air lock type of entry door. I think it is well worth considering having an automatic door closer.

HENKE: Yes, I have tried to emphasize the necessity of having air lock entry for all containment zones. They are recommended within a basic facility, too. The functional areas should be separated by at least one set of doors.

NIMS: I think the same holds true whether it be in laboratories or animal rooms. One contingency that is too often neglected is what I call disaster planning. Regardless of the other hazards in the laboratory, a fire can be a real problem. Eye washes and safety showers are not good unless they are tested, and it is interesting to speculate how many people know where the fire extinguishers and safety equipment are in their laboratories.

WOODS: I am Jim Woods from Iowa State University. I have several questions for Dr. Barkley and one for Mr. Henke. In comparing the mass airflow system to the conventional system, was anything indicated about methods of air diffusion within a conventional system? What technique was used to locate the diffusers and what kind of filter efficiency was used in the conventional system?

BARKLEY: I am sorry that I did not make that clear. Those were calculations of expected contamination levels from a continuous source of 10,000 particles per minute, assuming perfect mixing with a continuous source. They were not the results of any particular study.

WOODS: Mr. Henke, you mentioned some halide testing for leak-test validation of your rooms. I wonder if you would elaborate a little on how you challenge the system so you can use the halide tester and what the results have been.

HENKE: The halide test has only been used in a few prototype laboratories that were developed for the National Cancer Institute and, more recently, for one of the containment laboratories set up at the Center for Disease Control. It is a very qualitative test. What we do is establish the room under the normal operating negative pressure. Someone outside sprays freon over the joints and surfaces of the room, while another person follows inside the room with a halide torch. If there is a leak, the freon will diffuse in and it will be detected by the halide torch.

WOODS: Have you ever used sulfur hexafluoride?

HENKE: No, we have not.

WOODS: I do not know a lot about that particular device except that the National Bureau of Standards is having quite a bit of success in using it as a challenge because they have developed some very sensitive instrumentation.

HENKE: I think that is very important. There is a deficiency in our spectrum of test methods between the crude, soap bubble type of test and the halide test. We do not have good instrumentation or methods within that range. I would like to see some developed, because testing facilities would be easier.

MORELAND: I am Dr. Moreland from the University of Florida. Dr. Barkley, you stated that you were not recommending filtration systems for moderate-risk facilities. Looking over the list of organisms given to us by Dr. Gerone, I see that resistant organisms and spore-formers, such as *Bacillus anthracis* and *Mycobacterium tuberculosis*, are placed in the moderate-risk category. Certainly, we must face the fact that these agents could be transmitted through the effluent air and spread around the community. Although your studies showed that no outbreaks or epidemics have been traced to faulty filtration, it is still a risk and we must be concerned about it. I realize it would be costly to install filtration systems in all these facilities, but I wonder if the risk does not still necessitate it.

BARKLEY: I appreciate the question, and it gives me an opportunity to acknowledge the late Dr. Wedum, who identified a number of laboratory epidemics in some of the data that I presented. It is true that, under conditions in which we are working with moderate-risk agents, the

opportunity exists for them to escape from the laboratory or the animal room. However, it is important to realize that, in all the epidemics that have been described, no control conditions were in existence, on the one hand, and very little attention was paid to safety, on the other. If an agent with which one is working represents a potential risk to the community from an accidental discharge in the general ventilation system, it is an agent that requires more containment than we generally enforce at a moderate-risk level.

Further, there have been cases of secondary infections involving a contact between a laboratory worker who had been exposed and became diseased and someone in the same household. If the hazard is great enough for us to go to the extent of treating the general exhaust air, the air we breathe when we occupy those facilities, then we need to provide additional measures to protect the community from the laboratory workers once they leave the facility. Emphasis needs to be placed on primary control measures and the response needs to be made at the immediate time of an accidental or inadvertent exposure so no dissemination will take place. The evidence does not suggest that we need to go to the extent of exhaust air treatment for moderate-risk agents, although political and public pressure will force us to do so. Also, the laboratory worker will tend to ask for and accept any engineering containment feature that will not inhibit his or her work on the bench. I will support people who want to put filtration systems in, but I also want to put in clothes-changing procedures as well. We must be consistent.

EKSTROM: I am Dr. Merlin Ekstrom from the Division of Laboratory Animal Resources at Wayne State University, Detroit, Michigan. This morning, many very important safety practices relating to containment of hazardous agents in the biomedical research laboratory and animal facility environment have been discussed. Various federal agencies have prepared excellent publications devoted to these topics. Some of these publications have been referred to this morning. However, these are difficult to obtain without knowning publication numbers or costs. Has a list of these documents been compiled?

FOX: Yes. If you will write to me, I shall send you a list. My address is:

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IV

Cost-Effectiveness

Opportunities for Energy Conservation in Animal Laboratories

LAWRENCE G. SPIELVOGEL

Laboratory facilities by their very nature are energy-intensive. Compared with typical commercial buildings, such as office buildings, they can use 10-30 times as much energy per square meter. I will explore the reasons why laboratory facilities are so energy-intensive and present means for improving the energy efficiency of these buildings.

DESIGN CONSIDERATIONS

Design considerations for energy conservation in laboratory facilities begin in the first stages of planning and programming, where programming is defined as the setting out of requirements for a new facility. The concern for saving energy must be expressed as a consideration at the same time as the requirements for space and utilities are being planned.

The relationship of spaces in a building and the mechanical systems that serve them will determine energy consumption to a much greater degree than just about any other feature in a building (Spielvogel, 1976a). As an example, if only 10 percent of a laboratory needs to operate 24 hours a day, yet the entire facility is served by one air-handling system, the remaining 90 percent of the facility will be comfort-conditioned and ventilated during times when that is not required. In many buildings, this period can run as much as two-thirds or three-quarters of the total time, thereby amounting to considerable wasted energy. The use of the facility must be examined carefully before it is designed. How much flexibility is needed in terms of hour-by-hour, room-by-room use? Will all rooms be in use simultaneously? Will some rooms not be used for days, weeks, or months at a time? Since hours of use are the primary determinant of energy consumption, the most serious consideration must be given to segmenting the uses in a building and providing separate comfort-conditioning systems that can be operated only when necessary.

Setting forth the comfort-conditioning requirements for space also has a substantial potential influence on energy consumption. The requirements for temperatures, humidities, pressures, and ventilation must be thought out carefully for each and every space in the building. All too often designers assume that these items will be uniformly necessary throughout the entire building, thus simplifying design, but frequently creating an energy hog. Although the need for controlling temperatures individually in various spaces throughout a building may not in itself contribute to high-energy consumption, when combined with requirements for humidity control, pressurization, and ventilation the type of mechanical system selected to meet these other requirements can become energy-inefficient. It, therefore, becomes imperative to examine closely the requirements other than temperature that are to be imposed on the heating, ventilation, and air conditioning (HVAC) system.

How precisely must humidity be controlled? Must relative humidities be kept below or above a certain level all the time? Must it be the same in all spaces? For what reason is humidity control necessary? What tolerances are allowable? Can individual dehumidifiers in each space or group of spaces provide the necessary control? Is pressurization required to preclude the flow of odors and contaminants from one space to another? Must pressurization be provided by the HVAC supply system, or can it be handled by exhaust systems? Do the pressurized areas have to

be in the same structure as unpressurized areas, or would it pay to build a separate wing or building to house them? How much ventilation is necessary? Is it necessary in all spaces to the same degree? Could odors or contaminants be eliminated in other ways? Can makeup air for hood exhausts be supplied separately?

Answering these questions will give the designer the information necessary to select the mechanical systems that will meet the needs of each space in the building. One minor unnecessary requirement can cause the selection to shift to an energy-intensive system. For example, requiring that the relative humidity never exceed 50 percent will probably lead to the selection of a reheat type of system, generally, although not always, the most energy-intensive (and the most common) system that can be used.

The type and number of systems selected to meet these requirements will also have an important bearing on energy consumption. There is nothing wrong with having many different types of systems to serve many different types of requirements, especially if each system is selected to do the necessary job at maximum efficiency. It may complicate the building and the design, but the results will frequently be worthwhile in terms of operating costs. Finally, the ease with which the HVAC systems can be controlled and operated will help to determine energy consumption of a building. The inclusion of controls, switches, and time clocks accessible to the users of the building is the key consideration. Even the most inefficient system that can be turned off when not needed will use less energy than the most efficient system that must remain on for more hours than necessary. Because the users of the building determine the need for climate control, they are the ones that control the ultimate use of energy (Spielvogel, 1976b); therefore, they must have the necessary and reasonable means for operating with energy in mind.

FACILITY COMPONENTS THAT USE ENERGY

The principal components that use energy are heating and cooling systems, fans, lights, and equipment. It is helpful to think of these components in planning the design and operation of a laboratory facility and in evaluations of reasons for energy consumption in existing laboratory facilities. When one looks to reduce energy consumption, these are the major components to be addressed. When the energy consumption for these components is measured or can reasonably be determined, it will give a good indication as to what is and is not worth attacking. Designers have traditionally been concerned with ensuring that equipment with adequate capacity is specified to meet even the most extreme conditions that can be encountered. They have similarly been concerned with the energy efficiency at these conditions. However, both in theory and in practice, extreme conditions are encountered only a few hours per year. During all other hours the building operates under part-load conditions for which efficiency is not specified and performance data are not readily available.

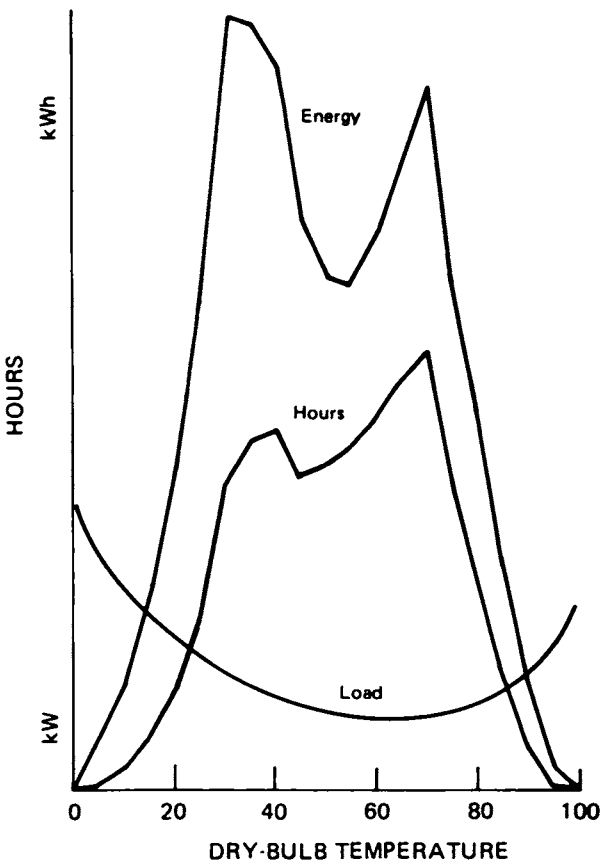

FIGURE 1 Relation of loads to energy consumption. Graph courtesy of L. G. Spielvogel.

Figure 1 illustrates the climate control loads for a hypothetical building in Washington, D.C., as a function of temperature. Hours of temperature occurrence in 2.8°C increments were obtained from U.S. Air Force weather data (U.S. Air Force, 1967). Annual energy consumption is the product of hours and load.

Almost two-thirds of the annual energy consumption occurs at temperatures between -1.1°C and +21.1°C. During all these hours, the systems are operating at less than half of their rated capacity. Therefore, one should be more concerned about how energy-efficient the systems and the building are under these "moderate" temperature conditions than at the extremes (Spielvogel, 1974).

ACTUAL ENERGY USE IN A LABORATORY

Table 1 compares the actual energy use in a 3,000 m^2 research laboratory with the energy use in a "typical" office building. This laboratory is located in Pennsylvania and has been in operation 24 hours a day since 1973. Most of the building is provided with 20 air changes per hour of 100 percent outside air through a terminal reheat system with humidification. Certain selected rooms have supplemental humidifiers to maintain

TABLE 1 Comparison of Energy Used in a Research Laboratory and Office Building

Component	Million J/m²/yr Research Lab	Office Building
Fans, pumps, and miscellaneous	9.50	1.42
Lights	6.46	2.85
Air conditioning	4.37	0.95
Hot water and reheat	11.01	0.38
Heat and humidification	9.50[a]	2.66
TOTAL	40.84	8.26

[a]With heat recovery--amount could be 31.7-42.2 million J higher without heat recovery.

higher conditions. Heat recovery is accomplished by a run-around system from exhaust to supply and a mechanical system for cooling the exhaust air when additional heat is required. Supply and exhaust systems are intentionally unbalanced to provide three levels of pressurization--this precludes the movement of odors and contaminants from room to room. Air supplied to the corridors is exhausted through the service areas to provide some cooling.

It can be seen that, even in this reasonably energy-efficient laboratory, the energy consumption is five times as much as in a typical office building. Energy consumption in other types of laboratories can go as high as 142 million J/m² a year. The National Bureau of Standards publishes data on its energy consumption and shows that, even with an energy-conservation program in effect, it is in excess of 95 million J/m² a year.

The reasons for the energy intensiveness of the various components can be attributed directly to the functions and conditions required in the space. Fans and pumps use considerably more energy in labs than in office buildings because of the longer hours of operation and the fact that more air is handled per square meter. Energy consumption for lights is higher in almost direct proportion to the hours of use and to the lighting intensity. Energy used for air conditioning is greater in laboratories than in offices because of the longer hours of operation and the need to cool much larger quantities of outside air. The strikingly higher energy consumption for hot water and reheating stems from the constant use of hot water in some laboratories, the the need for hot water to wash materials and equipment, and the need for reheating to control temperature and humidity. The higher requirement for heat and humidification in laboratories is attributed to longer hours of operation and higher quantities of ventilation. Since the cost of energy is on the order of $1.00/1 million J, it can be seen in this example that the annual energy cost for laboratories can run at least $40-$50/m². Because the annual cost of operating a research laboratory per dollar of construction cost is considerably higher than for most other buildings, a climate is created in which energy-conservation measures generally appear more attractive than in most other types of buildings.

EFFECTIVENESS OF HEAT RECOVERY

There has been a proliferation of heat recovery equipment and systems on the market. Manufacturers claim that heat recovery efficiencies in the range of 60-80 percent are attainable. The American Society of Heating, Refrigerating and Air-Conditioning Engineers is developing standards by which manufacturers' claims can be evaluated and compared.

Substantial differences can be measured between the design-rated efficiency of a heat recovery device and its seasonal efficiency. A great deal of this difference is attributed to the type of building and HVAC system to which the heat recovery device is applied. It is not at all uncommon to find that the seasonal efficiency of an 80-percent-efficient heat recovery device is only 40 percent. The principal reason for this disparity is that not all of the recovered heat may be necessary in the building, especially during moderate weather.

COMPUTER SIMULATION

Hour-by-hour computer simulation is being used to analyze the energy consumption of existing and proposed facilities. The major advantages to computer simulation are the abilities to handle complex situations, evaluate many options, weigh concepts that could have positive and/or negative energy impacts, and predict the relative magnitude of the energy use.

Once a simulation of a building has been made, it becomes a simple matter to evaluate any number of variables, such as changes in control settings, occupancy, equipment, and performance (Spielvogel, 1975). The results may then be used as the basis for the design of a new building or for changing the operation of existing buildings.

LIFE-CYCLE COST

Life-cycle cost is the latest buzz word in building design and operation. The term implies taking into account both the initial cost and the projected cost to be incurred during the life of the building. It is generally invoked to justify a more costly initial installation on the basis of a lower long-term cost. Life-cycle costing is being touted as the ultimate way to achieve energy conservation. However, there is no fundamental relationship between energy and life-cycle cost. Table 2 shows a 30-year life-cycle cost analysis for six systems in an anonymous corporate facility. Table 3 shows the same figures shifted in a way that all cost items are shown as increments to the lowest cost in each category. Table 3 discloses that

TABLE 2 Summary of Life-Cycle Costs

System Number	First Cost, $	Annual Energy Cost, $	Life-Cycle Cost, $	Energy Budget, million J/m^2/yr
1	0	349,300	13,178,295	9.36
2	+75,000	345,209	13,139,615	8.96
3	+125,000	340,271	12,534,528	5.55
4	+325,000	335,165	12,720,649	5.17
5	-50,000	357,587	12,643,621	6.75
6	+75,000	338,111	12,916,086	8.04

TABLE 3 Summary of Shifted Life-Cycle Costs

System Number	First Cost, $	Annual Energy Cost, $	Life-Cycle Cost, $	Energy Budget, million J/m^2/yr
1	+50,000	+14,135	+643,767	9.36
2	+125,000	+10,044	+605,087	8.96
3	+175,000	+5,106	0	5.55
4	+375,000	0	+186,121	5.17
5	0	+22,422	+109,093	6.75
6	+125,000	+2,946	+381,558	8.04

the system that has the lowest life-cycle cost does not have the lowest energy consumption. Similarly, the system with the lowest first cost does not have the highest energy consumption. Moreover, even energy cost is not proportional to energy consumption. Were any of the life-cycle assumptions, such as the life-cycle itself, the tax structure, or the rates of inflation and escalation, to be changed, the life-cycle costs would change, and, in all probability, the relative ranking of the systems would change. Therefore, it is suggested that life-cycle costing be used with reservations based on the sensitivity of its elements; it should not be relied on as an absolute technique for decision-making.

AMERICAN SOCIETY OF HEATING, REFRIGERATION AND AIR-CONDITIONING ENGINEERS (ASHRAE) STANDARD 90-75

ASHRAE Standard 90-75, *Energy Conservation in New Building Design* (ASHRAE, 1975), was published in the fall of 1975 and is being widely used to set at least minimum requirements for energy-efficient design. All or parts of it have been included in some statewide building codes and model building codes. Therefore, it will apply to the design of new laboratory facilities. Laboratory facilities are exempt from certain portions of the requirements of ASHRAE Standard 90-75, such as those for HVAC systems, thereby permitting the necessities of the particular laboratory to dictate HVAC system selection and design. This exemption makes it incumbent upon the user and designer to be most careful in their setting forth of design criteria and selection of energy-efficient systems.

CONCLUSIONS

In the design of future laboratories, plans for temperature, ventilation (or number of air changes), pressurization, and humidification must be examined more closely. Since these elements determine the type and number of HVAC systems, they should be set at requirements that provide for minimum energy needs. The use of heat recovery systems will probably become commonplace in laboratory facilities. Various functions should be segmented and separate systems provided for each of them. Time of operation is the principal consideration. Finally, efficient means of operating the building and its systems must be provided. These means entail the use of controls that permit the user to operate only what is needed, where it is needed, when it is needed.

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Discussion

WOODS: Jim Woods, Iowa State. Mr. Spielvogel, you mentioned annual energy values that ranged up to about 142 million joules per square meter, I believe. Can you make a bold estimate of a reasonable budget for a laboratory animal facility?

SPIELVOGEL: I am sorry, I hesitate to do that. The reason I hesitate to even recommend a particular goal or state a particular ballpark figure is that when we look at an actual building, such as an office building, we find energy budgets that range from as low as 3.8 to as high as 52.8-76.0 million joules per square meter a year. These are relatively uniform buildings, whereas most laboratory animal buildings are very specialized. Hundreds of office buildings have been measured and reported, but this is the only laboratory that I have ever seen measured and reported. With the wide range of values found in office buildings, I would have to see a lot more laboratories before I could even think about saying what kind of energy budget might be established.

Energy Conservation in Water Heating and HVAC Systems

ROBERT L. GORTON

The primary opportunities for energy conservation in laboratory animal facilities exist in the service water heating system and in the heating, ventilating, and air-conditioning (HVAC) system. Intelligent design of these systems, with reduction of energy requirements as a goal, can result in cost-effective systems with no disturbance in the level or quality of service provided. Initial investment in equipment may be high. However, the necessity of providing service on a continuous 24-hour day, 7-day week basis should recover that investment through reduction in energy costs.

HVAC SYSTEM LOADS

A profile of a HVAC system load, typical of what might be encountered in a laboratory animal housing facility, is shown in Figure 1. The relative magnitudes of the load components indicate that the ventilation load is the major element over which control may be exercised. Loads from lighting and animal metabolic heat are also large, but these are generally a function solely of amount of facility use, and no control is possible at a given level of activity in the facility.

Calculations indicate that ventilation loads typically are in excess of 50 percent of the total load at maximum summer design conditions (Gorton, 1975). Of this total, there is approximately a 40:60 ratio of sensible to latent load. The large ventilation load is caused by the requirement for large quantities of outside air for decontamination and odor control in the animal housing facility. The load may be reduced by treating and recirculating a fraction of the room air or by using heat recovery devices in the ventilation air system.

Success in reducing or eliminating airborne infection by laminar airflow and mass airflow rooms with filtered recirculation has been reported (McGarrity and Coriell, 1976). However, energy costs for recirculation fans are greatly increased. These costs may be offset by less energy used for temperature and humidity control in facilities using conventional ventilation. Despite reported success in buildings in which research is conducted, recirculation of room air has apparently not received wide acceptance by animal facility operators. Filtration systems have not proven totally effective in controlling sanitation in animal housing areas. The causes for the reported lack of effectiveness seem related, at least in part, to lack of vigilance in maintaining the system.

HEAT RECOVERY DEVICES

The second option, installation of heat-recovery devices, is a candidate for application in any situation in which the ventilation rate is high. Equipment for recovering heat from ventilation air is well known to engineers and has been described in the engineering literature (Bowlen, 1974; Gorton, 1975; ASHRAE, 1976). However, such devices are perhaps not so well known to animal facility operators who are potential users, and thus they are briefly described here.

These devices fall in five primary categories: run-around systems, heat pipes, plate-type recuperators, rotary exchangers, and desiccant-spray systems. With all of these, energy is exchanged between two points in the system. For cooling service, the warm, humid outside air discharges energy to the cooler system exhaust air. For

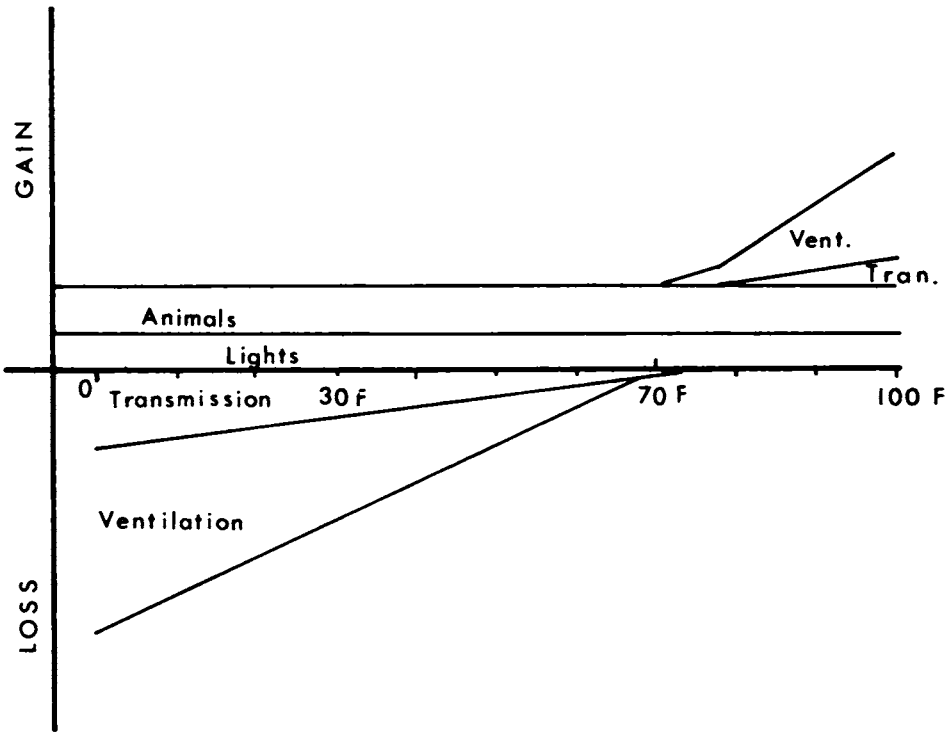

FIGURE 1 Typical load profile for a laboratory animal facility. Graph courtesy of R. L. Gorton.

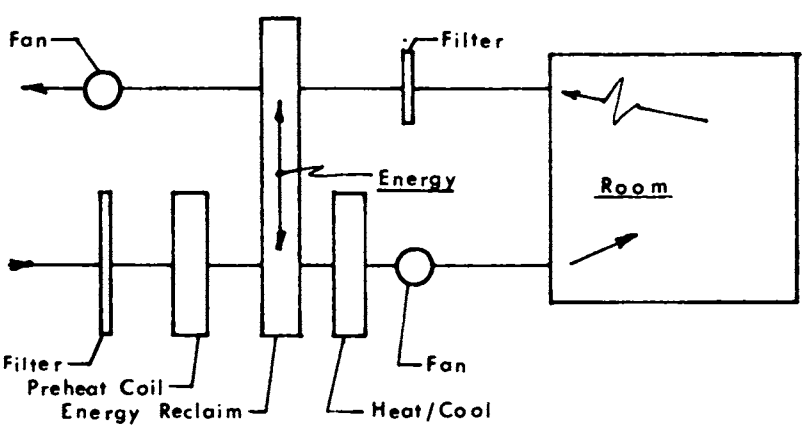

FIGURE 2 A heat recovery system using ventilation air. Diagram courtesy of R. L. Gorton.

heating service, the warm exhaust air loses energy to the incoming cold air. A typical arrangement of components is pictured in Figure 2.

Run-Around Systems

If a run-around system is used, conventional heating/cooling coils are inserted in the intake and exhaust ducts. The coils are connected through a closed loop, which includes a pump. In the summer, the circulating fluid is heated in the intake duct coil by warm intake air. The fluid is pumped to the exhaust air coil, where energy is transferred to the cooler exhaust air. The cooled fluid is then directed back to the intake coil, where it again receives heat, cooling the intake air. For winter operation, the cycle remains the same, but the heating/cooling functions of the two coils are reversed. This system has advantages in that it is controlled simply and automatically by pump speed or a liquid line control valve and because of the possibility of the two coils being physically remote from each other. The flexibility of the second possibility allows versatility in equipment arrangement and conceivable savings in construction costs.

Heat Pipe Systems

The heat pipe consists of a tube fitted internally with a screen, other porous wick material, or a grooved inner tube wall surface. During construc-

tion, the tube ends are sealed, air is removed, and a small quantity of one of various liquids is introduced. The liquid fills the pores in the wick. In operation, one end is heated and the liquid vaporizes and moves from the wick to the center of the tube. Capillary action then draws more liquid through the wick to the heated section. The vapor flows to the other end of the tube, where heat is removed. This process results in vapor condensation, the condensate replenishing the liquid that has migrated because of the wicking action. This cycle of operation continues as long as a temperature difference exists between the two ends. The vapor evaporation-flow-condensation sequence allows extremely high rates of heat transport to be maintained between the evaporator and condenser section of the heat pipe. Heat pipes are arranged in banks resembling conventional finned coils.

No controls are necesary for operating heat pipe units, as they function solely when a temperature difference exists between the two ends, the rate of heat transfer being determined by the magnitude of the difference. No seasonal changeover is required, because the evaporator-condenser section functions are reversible, depending only on the direction of the temperature difference. No external pump is required as the unit "pumps" the fluid internally. The intake and exhaust ducts must be adjacent to each other, which may take some flexibility away from the design.

Plate-Type Recuperators

These devices consist simply of a series of corrugated sections through which air flows. The sections are separated by solid-plate flow dividers. Alternate sections may be oriented at right angles, such that the two air paths are in a cross-flow arrangement. Heat flows through a convection-conduction mechanism from one fluid to the other. Because of the extensive surface area provided by the corrugated sections, the effectiveness of the heat transfer can be quite high.

Rotary Exchangers

The rotary exchanger is typically a densely packed wheel of corrugated metal or other porous material. The wheel turns alternately through the intake and exhaust ducts, picking up energy from the warmer stream and delivering this, as it cools, via the cooler stream. The exchanger is controlled by the rotational speed of the wheel. Adjacent intake and exhaust ducts are required. A potential for carry-over of pollutants from the exhaust to the inlet stream exists, but it has been minimized by design modifications.

The effectiveness of all the devices discussed above is in the 50-80 percent range at design operation. Effectiveness is usually defined as

$$\eta = \frac{\text{actual heat transfer}}{\text{maximum possible heat transfer}}$$

or, in many applications,

$$\eta = \frac{\text{actual } \Delta T \text{ of one stream}}{\text{maximum } \Delta T \text{ between the two streams}}$$

The heat transfer is the sensible, or temperature-dependent, heat transfer. Recall that approximately 60 percent of the design ventilation load (30 percent of the total load) may be latent heat. This portion is not available for recovery in the sensible heat devices that have been presented. However, the rotary exchanger (and the desiccant-spray system discussed below) may be designed to transfer water vapor, as well as sensible heat, from one stream to the other. In the rotary exchanger, vapor is transferred by coating the heat-transfer surfaces with a desiccant material. This material absorbs water vapor from the surface and delivers it, by desorption, to the lower-humidity stream. This mass transfer is equivalent to latent heat exchange. Such a system then has a potential for recovery of an additional 30 percent of the total load.

Sprayed-Desiccant Systems

In sprayed-desiccant systems, exhaust air is passed through the spray, giving up heat and water vapor to the desiccant solution. The solution is pumped to a second unit, where it is sprayed over the incoming ventilation air, warming and humidifying it. For summer operations, the airflow paths remain the same, but the exhaust air acts to cool and dehumidify the solution, which is then pumped to cool and dehumidify the incoming air.

All Systems

Each system described above has the capacity for recovering an appreciable fraction of the energy contained by the exhaust air for use in conditioning the intake air. It is obvious that each system must also be provided with sufficient heating/cooling power to supplement whatever capacity it may have to recover a portion of energy.

There is a natural desire to ask that one of the above-described systems be designated as "best" for the animal-room ventilation system. This labeling is not possible, however, because the "best" system for a particular application depends on geographic location, energy source(s) and cost, space available for equipment and ductwork, and maintenance and service considerations. A few features of various exchangers can be emphasized and their influence on system selection can be recognized. If intake and exhaust ducts are not adjacent, and rerouting is not possible, it will be necessary to use the run-around or the sprayed-desiccant type system; if latent loads are high (as they would be in areas with humid summers or dry winters), those systems that permit humidity as well as heat transport deserve special consideration. Beyond such obvious statements, however, generalization is not valid and job-specific considerations will dictate proper design.

It should be noted that the design is not a matter of simply choosing the most efficient unit available for the price. Because savings are

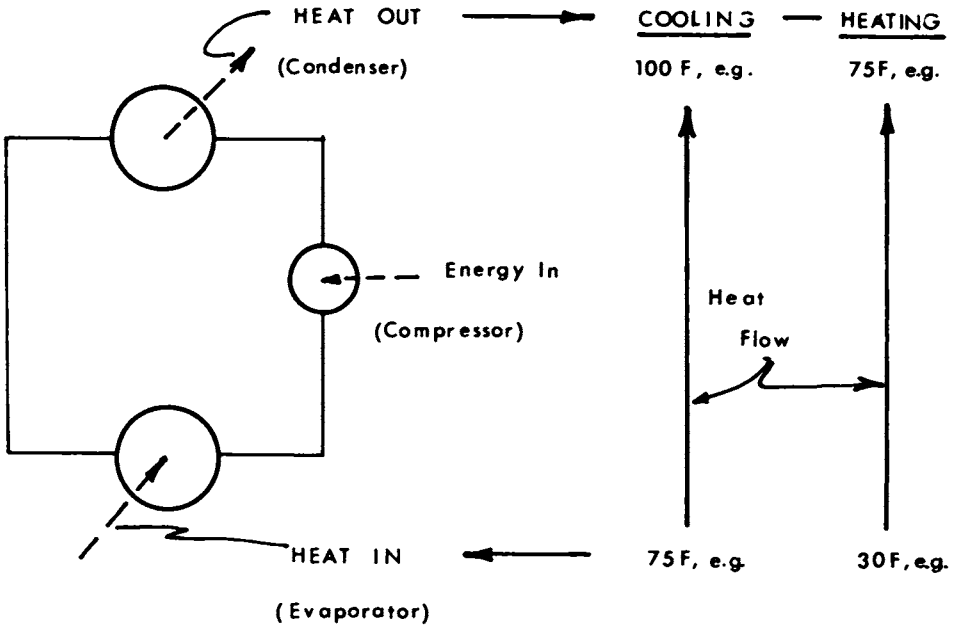

FIGURE 3 How a heat pump operates. All temperatures are merely examples for purposes of illustration. Diagram courtesy of R. L. Gorton.

fewer when outdoor temperatures are nearer the indoor design temperature and because more efficient units are generally more expensive to operate, it is necessary that a thorough engineering and economics study be performed to aid in the wisest selection of components.

OPPORTUNITIES FOR REDUCING ENERGY IN HEATING/ REFRIGERATION SYSTEMS

A refrigeration system is a device that accepts heat at a low temperature (in an evaporator) and rejects heat at a higher temperature (in a condenser), a process driven by an energy-addition component (compressor) in the cycle. If the device is arranged to provide both cooling and heating services, it is called a heat pump. Such a system is represented in Figure 3.

Some conventional refrigeration systems can be arranged to operate in a modified "heat pump" mode. This adjustment is possible with a split-bundle condenser, and such a system is very attractive in installations that require year-round cooling (as animal rooms do) as well as heating (service hot water, space heating in perimeter zones). This is illustrated in Figure 4, where in winter operation the energy removed

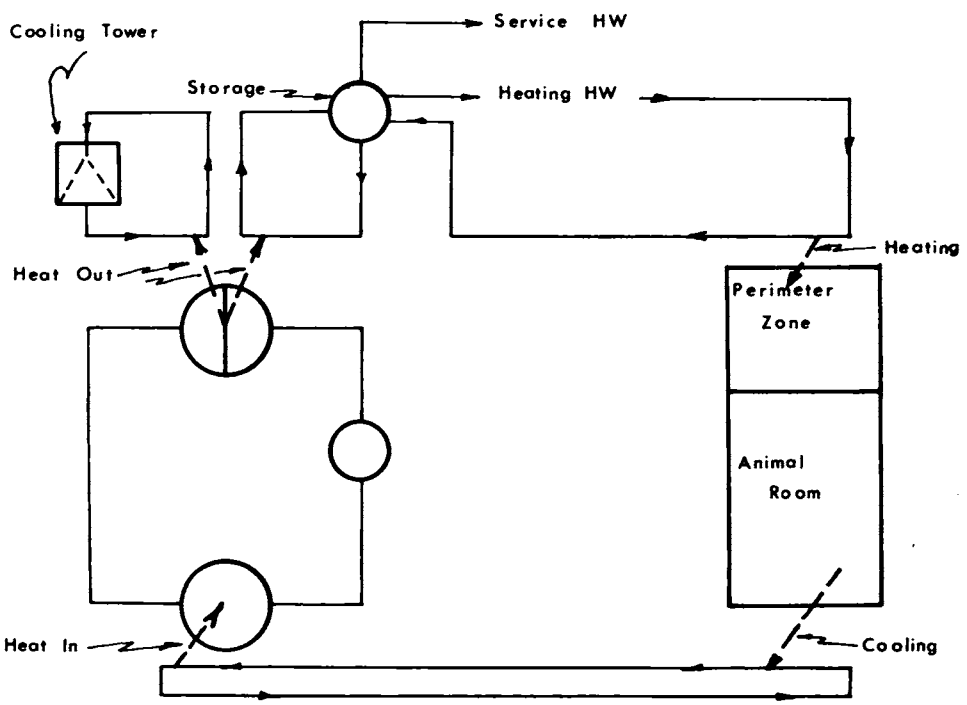

FIGURE 4 Split-condenser system; HW = hot water. Diagram courtesy of R. L. Gorton.

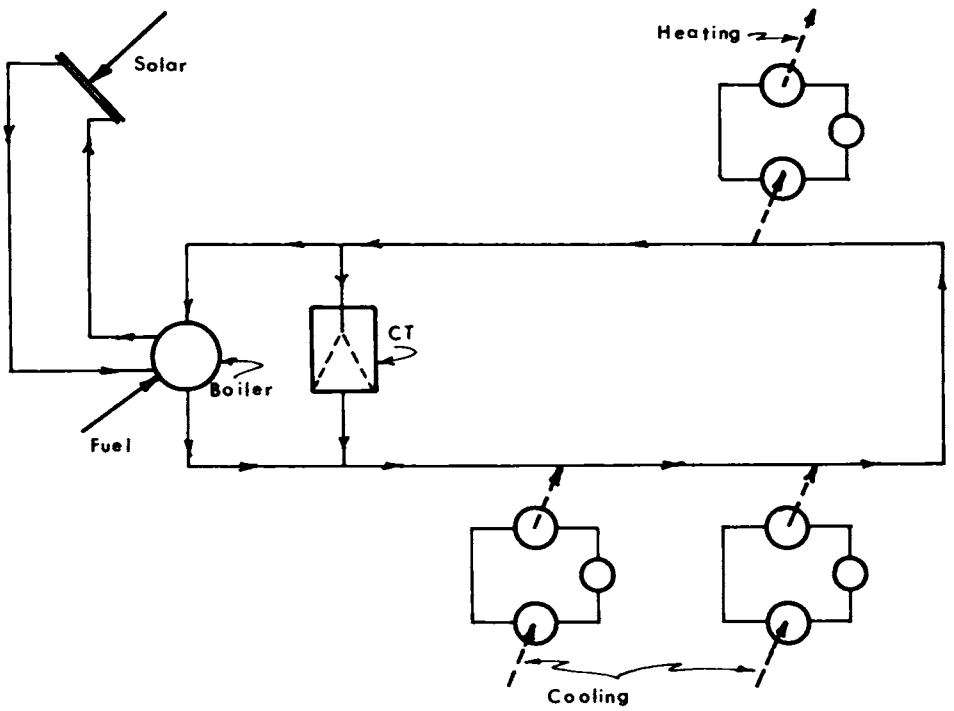

FIGURE 5 Heat pumps with common water loop; CT = cooling tower. Diagram courtesy of R. L. Gorton.

from the building core (cooling the animal room) is rejected to the hot water system and used for heating service water or for heating a building perimeter zone.

Energy may also be exchanged through unitary heat pumps connected to a common water loop, as diagramed in Figure 5. Here a cooling tower and a water heater are installed to maintain proper temperature levels in the water loop, either rejecting or adding heat as required by the total heating/cooling demand in the conditioned space.

When the system has become sufficiently complex to allow the flexibility described above, it will contain numerous heat exchangers, storage tanks, pumps, and controls. Once these elements have been installed, additional options, such as solar water heaters, windmills, and possible geothermal energy sources, become financially reasonable. Again, it should be emphasized that a system's technical and economic feasibility must be determined from detailed analysis by a competent engineer.

CONCLUSIONS

The energy-conservation systems described generally employ well-developed technology and components proved in the field. These systems and components have a relatively long history of use in specialized services. The prospect of continuing increases in energy costs has spurred their application to a more general service. It should be stressed that adoption of these methods depends on their economic, rather than their technical, feasibility. A very detailed financial analysis, considering all technical options, is required for proper system design and component selection.

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Energy Sources and Costs for Building Systems

FREDERICK H. KOHLOSS

Facilities for housing laboratory animals also include areas for storage, administration, surgery and necropsy, personnel lockers and showers, diet preparation, equipment cleaning, and incineration. The cost of energy is a significant portion of the total operating cost of such facilities. Rarely will an animal facility be sufficiently large or separated from related research or teaching facilities to dictate the selection of energy sources or to control an energy cost analysis by itself. Application of well-known thermodynamic principles to analysis of energy needs of a building can often lead to system designs that are energy cost-effective.

COMMON SOURCES OF ENERGY

Electricity

Usually provided by a utility or other off-site generating station, electricity may also be generated on-site and used for power and light. Direct use of electricity for heating is wasteful. Energy sources for utilities are not within the scope of this paper, nor is their efficiency of energy utilization. Electricity as one of the outputs of an on-site total-energy or selective-energy plant, or as the source of a heat pump, is energy-efficient and may be economical in many circumstances. Total energy plants are so designated because they have a fuel-fired, thermal, power-generation cycle, and the waste heat of the cycle is used as a source for heating and cooling buildings. Selective energy plants are similar, but they are operated in conjunction with electric utility service to take economic advantage of load variations. Such systems require large expenditures of capital and they are complex, having more operating and maintenance costs than systems with utility power.

Fossil Fuels

Natural or synthetic gas, if available, may be supplied to the site as a utility. Oil and coal may be purchased for storage and use on the site for generating power or as a source of heat. Laboratory animal facilities are usually part of a larger complex with standby emergency power, which can often be economical as a selective-energy or total-energy plant.

District Steam and High-Temperature Water

In some cities and on many campuses, steam is distributed, often at a fairly high pressure, and metered to buildings. To a lesser but growing extent, pressurized high-temperature hot water is being so used. Although steam may be employed in turbines to drive auxiliary equipment, its most common application is as a heat source.

Chilled Water and Brine

In a few cities and on many campuses, chilled water, or a solution with lower freezing temperature, is distributed and metered to buildings. It is a source of cooling.

Natural Energy Sources

There is a small yet growing use of solar energy as a heat source. Geothermal energy, wind energy, and municipal waste-burning plants are beginning to be contemplated or designed, and a very few are in use. Use of natural energy sources on-

site, as a means of conserving utility-provided energy and the on-site combustion of fossil fuels, is a key feature of energy conservation.

ENERGY FOR BUILDING SYSTEMS

Building systems are lighting, plumbing (including heating of hot water services), vertical transportation, materials handling, and air conditioning. The convenient distribution and utilization possible with electricity make it the only energy source usually considered for lighting, vertical transportation, and materials handling and the only power used for air conditioning fans and pumps. Occasionally steam, water, compressed air, or gas may provide limited power.

Animal facilities, like all other habitable buildings, need year-round air conditioning. Air conditioning includes heating, cooling, humidification, dehumidification, ventilation, and removal of air contaminants. In most climates, space heating is required to offset heat loss through the building envelope in colder weather and to warm the air brought in from outdoors. For small heating requirements, electric resistance-heating may be considered as a source, but this source may run afoul of energy-conservation regulations if used to excess. However, with certain larger loads, resistance heating is still economical. Heating may come from combustion of fuels to heat air or to heat a secondary transfer fluid such as water, which then heats air. Heating may also be accomplished by a refrigeration machine's heat rejection, in a heat pump. Waste heat or natural heat sources (such as the sun and geothermal energy) must seriously be considered. It is often easier to provide service hot water from waste heat or natural energy than space heating or air conditioning, since storage capacity required for off-peak use is reasonable.

Cooling is most frequently accomplished by mechanical vapor-compression refrigeration. Electric motors are typically the power employed, but fuel-fired engines or steam turbines may also drive compressors. Cooling by absorption refrigeration is fairly common, and it is very often an economical means of using waste heat or natural energy. Absorption refrigeration is based on the affinity of a salt such as lithium bromide for water. Refrigerant water, under a high vacuum in a closed cycle, boils at low temperature (about 5°C), removing heat through heat-transfer surfaces from piped circulating chilled water at ordinary pressure, which is used for air conditioning. The water vapor is dissolved by a lithium bromide solution in an absorber. The solution is then pumped to a generator, where external heat is applied, boiling off the refrigerant water from the solution at a temperature of about 40°C. The refrigerant water is condensed by another circuit of water, which is reused after it has been cooled by atmospheric air to about 35°C in a cooling tower. The cooling-tower water also removes the heat of absorption in the absorber vessel.

The absorption-refrigeration cycle is driven by thermal energy (the heat applied in the generator), a thermodynamically lower grade of energy than mechanical or electrical energy. Thus, it is theoretically far less energy-efficient than a mechanical-refrigeration cycle. The prime importance of the cycle today is its ability to use energy available at temperatures as low as 80°C, making it handy for use with solar-heated water, exhaust steam, or many waste heat sources. If wasted heat energy can be used for cooling, then the absorption cycle's inefficiency is not a drawback to its use.

Mechanical engineers use "coefficient of performance" (COP) to measure the energy efficiency of cooling and heating systems. In consistent units, COP may be thought of as the amount of energy received where it is wanted, divided by the amount of energy that had to be expended to receive it. To yield a realistic estimate, the calculations of the energy expended must include all auxiliary power the system needs for fans and pumps.

A vapor-compression mechanical-refrigeration system used for cooling has high theoretical and fairly high practical COP values, as indicated in Table 1. The desired result is the removal of units of heat energy from the air, and the energy input into the system is for driving the compressor and auxiliaries. The same machine can be used for heating air: In that case, the compressor's driving energy is also usable. Therefore, its heating COP is higher, by a value of 1, than its cooling COP. Note that the refrigeration machine can remove heat from one air system while simultaneously adding heat to another.

Electricity used for heating air by a resistance heater has a COP of unity. COP values of absorption-refrigeration systems have a theoretical maximum of unity and are about 0.55 in practice, as shown in the table. When fuel is burned for heating, the practical COP (heat to the air) is about 0.60-0.75 times the heat of combustion.

HUMIDIFICATION AND DEHUMIDIFICATION

Humans, normally clothed, at reasonable rates of metabolic activity, are comfortable over a wide range of relative humidity (ASHRAE, 1974), about 20-80 percent year-round, assuming good air distribution, a mean radiant temperature close to the room air temperature, and a room air temperature not too far from 24°C. The exhaustive studies of human comfort leading to these conclusions cannot be compared with research on laboratory animals, because they cannot tell us as clearly when they are too hot or cold, dry, or wet. For experiments, laboratory animals may have to be kept at closely controlled conditions of temperature, humidity, and air motion. Agreed-upon optimal conditions for commonly used laboratory animals all fall within the narrow range of 21-23°C and 40-45 percent relative humidity (Runkle, 1964), although particular species appear to remain comfortable and healthy over greater temperature and humidity ranges.

TABLE 1 Coefficients of Performance[a]

Heating or Cooling System	COP
Theoretical Carnot refrigerator (5°C source, 40°C sink)	$\frac{5 + 273}{40 - 5} = 7.94$
Theoretical Carnot heat pump (5°C source, 40°C sink)	$\frac{40 + 273}{40 - 5} = 8.94$
Theoretical vapor-compression refrigeration cycle (5°C evaporator, 40°C condenser, saturation cycle)	= 6.9
Theoretical vapor-compression heat pump (5°C evaporator, 40°C condenser, saturation cycle)	= 7.9
Practical compression water chiller (cooling)	= 3.0
Practical compression water chiller (heating)	= 4.0
Electric resistance heating	= 1.0
Practical absorption system (cooling)	= 0.55-0.60
Efficiency of fuel-fired heating	= 0.60-0.75

[a]Energy usable for heating or cooling, divided by energy required to make it usable.

It is unlikely that wide variations in temperature and humidity would cause animal discomfort, considering the natural habitats of some of the animals and the probability of wide variation already having occurred without notice within cages of laboratory animals in some facilities. Some evidence exists that animals may be more susceptible to disease and have lower breeding rates at temperatures and humidities outside the recommended range (Runkle, 1964). This is a research question, and the engineer or administrator concerned with design and operation of animal laboratories should apply the best available data for the particular case considered. As with humans, the rates of change of temperature and humidity may also have an effect on animals.

Outdoor air used for ventilation must be humidified in cold weather and dehumidified in hot weather other than in desert climates to keep indoor relative humidity stable and at an optimal level. Evolution of water vapor from animal respiration, excretion, and cage washing imposes a dehumidification load on the air conditioning system, year-round (Gorton and Besch, 1974). This mathematical model study indicated that cage washing will cause a shift in the room conditions from 23.9°C and 50 percent relative humidity (12.8°C dew point), to about 22.2°C and 77 percent relative humidity (17.8°C dew point) for up to 2 hours. Increased ventilation rate, floor heating, or additional dehumidification may be needed if the changed conditions are intolerable for the experiment. The air conditioning system controls will have to be coordinated with the washing.

Premises are usually humidified by introducing steam into the air, spraying warm water into the air, or evaporating water from a heated surface into the air. Dehumidification is accomplished by using refrigeration to cool air below its dew point, or by permitting absorption or adsorption agents to contact the air, from which they remove the moisture without cooling. Sorption is usually more economical if unusually dry air (low dew point) is required. In general, refrigeration, which dehumidifies while cooling, tends to be used in animal environments. Because overcooling is often required to achieve sufficient dehumidification, reheating is often needed with refrigeration. With sorption, heating is needed to dry out or regenerate the sorbent material. For both reheat and regeneration, use should be made of waste heat if possible.

AIR SYSTEMS

All air conditioning systems' effectiveness ultimately depends on the air-distribution system. It is outside of the scope of this paper to favor or oppose recirculation of air from laboratory animal rooms. It is generally agreed that relatively high room air-change rates are essential. Outdoor air is usually considered the best agent for odor removal, by dilution ventilation, and acceptable outdoor air quality has been defined (ASHRAE, 1973). If outdoor air has too much particulate matter, it must be cleaned or filtered; and, if it has too much dirt, aerosols, or odor-forming components, it may have to be washed or deodorized with an adsorbent such as activated carbon. Wherever activated carbon is used for odor control, provisions must be made for replacing the carbon when it has become saturated with adsorbed material. Air must be prefiltered efficiently to remove dust before it passes through activated carbon (Barnebey, 1958).

A particular requirement for laboratory animal facilities is that all locations of outdoor air

intakes and exhaust air or combustion stack discharges must be selected very carefully by a competent engineer familiar with the complications created by natural airflow around buildings. Exhaust systems also may require filtration or odor removal. Contamination of air intakes by reentry of discharged air or stack gases is all too frequently encountered. In quarantine facilities, extreme danger to personnel can result.

Because air-distribution systems operate year-round in laboratory animal facilities, the fan horsepower accounts for an appreciable proportion of the air-conditioning system's energy use. Fan horsepower increases with quantity of air circulated and with the square of the system's airflow resistance. Reducing the rate of air change in the laboratory animal rooms and allowing adequate space for simple air-duct systems and efficient apparatus configuration will save energy.

With caged laboratory animals, it is difficult to maintain the same environmental conditions in the cage as in the room (Woods *et al.*, 1975). Most frequently the animal cages are not directly coupled to the supply air, and the room air must be distributed in such a way as to provide the proper air distribution and heat transfer inside the cages. For axenic (germfree) animal housing or for quarantine areas, the cages may be coupled directly to the conditioned air supply; these are termed cage-coupled systems. Woods *et al.* (1975) derived mathematical models that predict steady-state temperature, humidity, and contaminant concentration for cage and room based on experimentally determined cage characteristics. Other papers on air systems design of laboratory animal facilities (Woods, 1975; McGarrity and Coriell, 1976) also deal with air distribution. McGarrity and Coriell demonstrated the effect of vertical air distribution of sterile air that had been filtered by a HEPA (high-efficiency particulate air) filter, from a perforated ceiling. Axenic mice in open cages 15 cm from cages of nonaxenic mice were maintained in the axenic state at fairly reasonable velocities, even when humans cleaned dirty cages. Their results confirm early experiments on the value of mass airflow distribution in controlling airborne spread of infection.

Woods (1975) defined a "system air distribution factor" as the actual air-change rate of a room-coupled cage compared to the expected rate determined in a reference system. The parameter called the air-distribution performance index (ADPI) is beginning to be used in air conditioning design. The ADPI is based on subjective human responses to drafts. Despite the presence of cages, acceptable ADPI values are obtained using an air-distributing ceiling. The effect of this air-distribution research will be to improve the performance of air systems in laboratory animal rooms and reduce the amount of energy they use by more efficient distribution of air.

COMPONENTS OF HEATING AND COOLING LOADS

Heat must be added to a laboratory animal facility to overcome building envelope heat loss to colder air outdoors, to warm the outdoor air introduced for ventilation, and to evaporate moisture to humidify that air. All other load components are year-round heat gains, and in cold weather they can reduce the building's heating requirements. In warmer weather, unfortunately, the heat gain components of lights and motors in the space, the heat flow from warmer air through the building envelope into the space, the solar heat gain, the people and animals in the space, and the too-warm and too-humid ventilating air all require energy for cooling and dehumidification.

Thus, for spaces on the building perimeter there may be a particular outdoor temperature at which heat losses balance heat gains. Even in winter, interior spaces may require cooling and dehumidification if a large number of people or animals, or other moisture sources such as cage washing, are present. With proper system design, this cooling and dehumidification can be accomplished by introducing cold, dry outdoor air without refrigeration or sorption. Good air-conditioning design can also minimize cold-weather energy requirements, because it is possible to apply heat removed from the interior areas toward offsetting heat loss to the building envelope. The ability of refrigeration equipment to cool one area while heating another is economically advantageous at this task.

Architectural design has, of course, a major effect on heating and cooling load. Considerations affecting energy conservation in new buildings are clearly outlined in ASHRAE Standard 90 (ASHRAE, 1975). This standard is not applicable to existing buildings, but ASHRAE has prepared a series of proposed standards for energy conservation in existing buildings, which are now in review and should be issued soon. Standard 90 suggests reasonable criteria for building envelope heat-transmission characteristics, air conditioning system features not unduly wasteful of energy, minimum performance standards for air conditioning equipment, a budget for lighting power use, and other items. It is a good basis for legislative control of energy waste in buildings.

The special temperature and humidity control requirements of laboratories are recognized in Standard 90, a good starting point for planning an energy-efficient facility.

CLIMATE EFFECT ON ENERGY SOURCES AND SYSTEMS

Often minimized in air conditioning system design is the economic effect of the local climate on the choice of an air conditioning system. For example, in some areas on the West Coast, the annual air temperature does not vary greatly, and the outdoor air has a relatively low humidity content in the summer. In contrast, in the Eastern United States, summers are extremely humid and greater temperature ranges are experienced. The lower winter temperatures act to increase the size of heating systems, and the energy consumed for heating the outdoor air, as well as for overcoming heat loss from the building envelope, is correspondingly greater. Higher outdoor humidity results in

TABLE 2 Typical Design Indoor and Outdoor Temperature,[a] and Energy for Conditioning Ventilation Air

Location	Winter Outdoor Design[b]		Heating Required[c]	Summer Outdoor Design[b]			Cooling Required[c]
	Deg. C[d]	Enthalpy (J/g Dry Air)	(J/g Dry Air)	Dry bulb (deg. C[e])	Wet bulb (deg. C[e])	Enthalpy (J/g Dry Air)	(J/g Dry Air)
Washington, D.C.	-8.3	14.12	45.42	32.8	25.0	94.36	34.82
Buffalo	-14.4	6.01	53.54	29.4	22.8	85.46	25.92
Chicago	-20.0	-0.67	60.21	31.7	24.4	92.04	32.50
Miami	8.3	43.45	16.09	32.2	26.1	99.13	39.59
Minneapolis	-24.4	-5.69	65.23	31.7	23.9	89.81	30.27
Kansas City, Mo.	-14.4	6.01	53.53	35.6	25.0	94.36	34.82
Denver	-17.2	2.61	56.93	32.2	17.8	66.45	12.09
Phoenix	1.1	29.27	30.27	41.7	23.9	89.81	30.27
Seattle	-3.3	21.67	37.87	30.0	17.8	68.17	13.63
San Francisco	3.3	33.31	26.23	25.0	17.8	68.17	13.63
Los Angeles	4.4	35.42	24.12	31.7	21.7	81.29	26.75
Honolulu	17.2	66.45	-6.91	30.0	23.9	89.81	30.27

[a] Adapted from *ASHRAE Handbook, Fundamentals Volume* (1977).

[b] Indoor design: 22.2°C, 45 percent relative humidity, enthalpy 59.54 J/g dry air.

[c] Heating and cooling per gram of dry air: For heating, the figure is the indoor air enthalpy per gram of dry air less the winter outdoor air enthalpy. For cooling, the figure is the outdoor air enthalpy minus the indoor air enthalpy.

[d] Taken as temperatures that are equalled or exceeded for 97½ percent of the total hours (2,160) in December, January, and February; there would be only 54 colder hours in the typical winter. Air is assumed to be saturated in listing its air enthalpy.

[e] Taken as the highest 2½ percent of all the hours (2,928) in June, July, August, and September (75 hours would be above the listed figure in the typical summer).

TABLE 3 Heating Season Temperature Variation, Annual Heating Celsius Degree-Days and Annual Equivalent Full-Load Hours[a] (Data Are Not Yet Available in SI Units and the Conversion Herein Is Approximate Only)

City	Hours per Year at Various Temperatures												Annual Heating Celsius Degree-Days[b]	Annual Equivalent Full-Load Cooling Hours[c]
	21.1 to 23.3	18.3 to 20.6	15.6 to 17.8	12.8 to 15.0	10.0 to 12.2	7.2 to 9.4	4.4 to 6.7	1.7 to 3.9	-1.1 to 1.1	-3.9 to -1.7	-6.7 to -4.4	Below -6.7		
Washington, D.C.	960	766	740	673	690	684	790	744	542	254	138	73	2,347	900-1,200
Buffalo	646	722	760	700	666	624	647	756	849	602	426	549	3,923	600-800
Chicago	762	769	653	592	569	543	531	800	822	551	335	497	3,419	700-1,000
Miami	1,705	810	452	277	147	71	26	4	0	0	0	0	78	2,200-3,200
Minneapolis	621	690	695	602	588	482	500	560	632	609	514	1,354	4,657	600-800
Kansas City, Mo.	761	723	601	572	553	562	628	625	591	407	265	350	2,617	1,200-1,600
Denver	549	684	783.	731	678	704	692	717	721	553	359	479	3,491	600-800
Phoenix	762	776	767	769	659	540	391	182	57	8	0	0	981	1,400-2,000
Seattle	258	448	750	1,272	1,462	1,445	1,408	914	427	104	39	23	2,458	800-1,200
San Francisco	285	665	1,264	2,341	2,341	1,153	449	99	10	0	0	0	1,667	300-500
Los Angeles	881	1,654	2,193	1,904	1,054	428	107	10	0	0	0	0	749	900-1,400
Honolulu	2,424	569	45	0	0	0	0	0	0	0	0	0	0	2,400-3,600

[a] From *ASHRAE Handbook, Systems Volume* (1976).

[b] For any one day when the mean temperature is less than 18.3°C, as many Celsius degree-days exist as the difference between mean temperature and 18.3°C.

[c] Data are subject to wide variation depending on the type of system and how the equipment is operated.

large cooling systems and sizable amounts of energy consumed for cooling and dehumidification.

Federal regulations and standardization policies frequently, usually unintentionally, fail to take climatic differences into account. Table 2 lists the wide climatic variation for some American cities and compares the energy required to condition outdoor ventilating air. Very roughly, 50 kg dry air and its associated moisture would have to be introduced per hour per square meter of laboratory animal-room floor area, as an indication of how a system might be affected by the table's figures. Obviously, the table gives the expected close-to-maximum figures used to size the system's equipment. In milder weather, less energy is required. For the same cities, Table 3 gives an indication of the temperature spread during the year, the severity of the heating season, and the estimated yearly equivalent full-load operating hours required by cooling equipment. Energy consumption calculations for air conditioning systems involving heat recovery must be more sophisticated than such rough data supplied in Tables 2 and 3 if they are to be good predictions of the matching and mismatching of cooling and heating energy demands during human working and off-duty hours.

LIFE-CYCLE COSTING

Rather than compare building systems solely on a capital-cost basis, it is of value to compare their total cost over their economic life. This is particularly important as inflationary pressures on operating costs continue. Laboratory animal facilities in particular should benefit from engineering attention to reduced energy consumption, even at higher installed cost.

PRACTICAL SUGGESTIONS

In planning and operating existing laboratory animal facilities, I make the following recommendations:

- Carefully establish the required indoor design conditions of temperature and humidity.
- Attempt to lower to a practical minimum the outdoor air ventilation rate.
- Analyze means of improving the cage-coupling to the supply air.
- Look for ways to use waste heat and alternate energy.
- Carefully analyze required lighting, using the lowest possible levels and most efficient light sources and luminaires.
- Check to see if an improved power factor can be achieved.
- Try to reduce peak electrical demand.
- Verify that the utility rate schedule being applied is the most advantageous.
- See if added insulation, shading of fenestration, or improved controls and monitoring can be installed economically.

Competent engineering analysis of energy sources and quantities required, based on accurate assessments of what constitutes the correct air conditioning system design for a particular facility, is essential in keeping energy cost at a minimum. Not only must the building systems design engineer consider initial capital cost limitations, necessity for simplicity of operation and maintenance, controllability, space and aesthetic limitations and compromises, and flexibility toward changes in usage, but he must have a clear understanding of the available energy sources and their advantages and disadvantages.

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Automated Systems

THOMAS E. HICKEY

Automated systems for laboratory animal care, including automatic watering, automatic flush caging, automatic dry-waste removal caging, central vacuuming, and automatic feeders have been available for several years. Advertising and sales promotions have claimed reductions in labor and maintenance requirements as the major advantages of these systems. However, little published information is available, particularly relating to the cost-effectiveness of these improvements.

Nielson (1970) detailed the economics of automatic flush caging, and a published seminar report by Altman and Hickey (1973) included personal experiences with automated animal caging. Allen (1973) discussed and compared some automated systems and provided cost and savings data from a manufacturer's viewpoint. Hickey and Tompkins (1975) described the effects of housing mice in automatic flush caging, but they did not present a discussion of economic factors.

In an effort to obtain current information on automated systems, I sent questionnaires to major users of these systems. Names of users were supplied primarily by leading manufacturers and included academic and governmental institutions, industry, commercial breeders, and private laboratories. The questions were:

1. What types of automated systems are you currently using?
2. What do you feel are the major advantages of each system?
3. What are the major disadvantages?
4. What types of maintenance costs are generated by your systems?
5. Have you performed cost analyses comparing your automated systems with more conventional or manual methods? If so, please enclose data.
6. Have you any other comments?

Although 32 institutions responded to the survey, the results are not necessarily representative of this total group. In general, comments on the advantages or disadvantages of an automated system (e.g., automatic watering) come from "2 or more respondents" and cost analyses usually are reported by only small numbers of respondents (3 for automatic watering devices; 5 for automatic flushing systems). If an automated system is widely used, these results may be very meaningful. If not, a certain amount of bias may exist in these data. The reader should interpret the findings accordingly.

AUTOMATIC WATERING

Automatic watering is the most popular automated system. Most of the respondents reported experience with it and submitted many helpful comments.

Description

The most common type of automatic watering system consists of a pressure reduction valve, filter, water lines, bleed-off valves and dispensing valves. The dispensing valves vary in design according to the species being watered. The water lines are usually constructed of plastic, copper, or stainless steel. This system can supply individual cages or mobile racks by a quick-disconnect coupler. Other systems include fountains and self-filling water bowls and troughs.

Advantages

The major advantages of automatic watering systems listed by two or more respondents, in order of frequency, were:

- labor or time savings;
- elimination of bottles, stoppers, and sipper tubes;
- more constant water supply;
- cleaner or fresher water supply;
- minimal maintenance costs;
- reduction of "weekend" work; and
- decreased risk of contamination.

Other advantages, listed only once, were: ability to medicate large numbers of animals from a single source, elimination of bottle washers, interchangeability of housing units, neater appearance of animal rooms, lowering of room humidity by eliminating dripping bottles, better visibility into cages, and minimizing drudgery of menial tasks.

Disadvantages

The major disadvantages of automatic watering systems listed by two or more respondents, in order of frequency, were:

- difficulty in measuring individual water intake;
- high initial cost;
- possibility of flooded cages from leaky valves;
- need for checking water valves;
- difficulty experienced by small mice in obtaining adequate water;
- need for maintenance of system;
- difficulty in adding medicaments to water;
- possibility that water supply may have to be treated to prevent mineral deposits; and
- tendency to overlook maintenance and take operation for granted.

Disadvantages listed by one respondent were: possibility of damage during transport of cages and racks, possibility of affecting many animals if water supply became unsafe or was shut off completely, difficulty in sterilizing the system by steam without special modifications, need for ascertaining that animals know how to drink from valves, and some decrease in observation of animals.

Maintenance Costs

Most of the respondents claimed to have small maintenance costs associated with automatic watering. Repair and replacement of parts (e.g., plastic water lines, dispensing valves, hoses) and replacement of water line filters were cited most often. Other factors included the time and labor to repair leaky valves, time required to flush the lines during rack cleaning, and time required to backflush sand filters.

Cost Analyses

Three respondents indicated they had performed cost analysis studies of their automatic watering systems. Five others were combined with automatic-flush caging and those findings are discussed in the section dealing with that subject.

Respondent A This respondent determined the approximate cost of labor and supplies for manual watering compared with the cost of an automated watering system. The results were presented as justification for the purchase of an automatic watering system and do not reflect actual experiences with the system. This evaluation was based on the average time required to water animals by hand in 11 buildings and 46 animal rooms during the 1973 calendar year. The following factors were used for calculating costs:

- Time required to water animals in each room as determined on a per unit (rack) basis. The time was calculated for:
  --routine daily watering, involving rinsing, refilling, and replacing bottles on the same cage. An average of 30.25 manhours/day was required during the work week (5 days):
  30.25 × 260 = 7,865 manhours/yr
  7,865 × $4.72 (avg. labor/h) = $37,122.80
  --routine weekly change of bottles. An average of 42.5 manhours/wk was required.
  42.5 manhours/wk × 52 = 2,210
  2,210 × $4.72 = $10,431.20
- Weekend work on an overtime basis. Three employees were required to water the small animals each weekend. They worked an average of 4 h/day:
  104 weekend days × 4 h = 416 weekend h
  416 × $4.20 = $1,743.00
  1,743.00 × 3 = $5,229.00
- Holiday work on an overtime basis (same as weekends). Eight holidays were observed:
  8 × 4 holiday h = 32 h/yr
  32 × $4.20 = $134.20
  134.20 × 3 = $402.60
- Approximately 30 minutes each day was required for washing bottles, stoppers, and sipper tubes:
  260 days × 0.5 manhours/day = 130 manhours/yr
  130 × $4.72 = $613.60
- Extra time required to water animals out of sequence. Newly received animals were disruptive to the working routine of the animal attendants. Calculating the time needed to water the new arrivals was difficult to determine. However, most animals arrived on 3 days of the week. Approximately 60 minutes was required each day:
  3 manhours/wk × 52 = 156 manhours
  156 × $4.72 = $736.32
- Equipment cost (breakage replacement):
  576 16-oz bottles
  288 8-oz bottles - $157.70

Total Annual Cost of Manual Watering = $54,693.22

Installation Cost of an Automatic Watering System

Pressure control and distribution systems for the described

Animal rooms	$14,373.00
Rack and cage systems installed	$44,338.26
Cost of automated watering	$58,711.26
Cost, including volume discount	$53,090.13

Respondents B and C Respondent B figured the initial cost of supplying 40 rabbit cages, 90 mouse cages, 175 rat cages, 14 cat cages, and 56 dog cages with bottles, stoppers, sipper tubes, and water bowls. Respondent B also included the annual salary of one technician, and these costs were compared with the initial costs of providing automatic watering for an equivalent number of animals.

The first year totals were $6,108.25 for the manual system and $6,825.00 for automatic watering. These figures do not include replacement costs for the manual system or labor costs for the automatic system. Respondent C reported that an automatic system saved the institution "84 labor hours per week for each 5,000 rodent cages, plus cost of operating bottle washer (steam, electric, clean uniforms, etc.)."

AUTOMATIC-FLUSH CAGING

Automatic-flush caging was the second most frequently listed automated system in the survey.

Description

Automatic-flush caging systems depend on moving water to flush excreta to a drain. There are two basic types: cascade flush and front-to-back flush. The cascade-flush system employs a series of stainless steel pans under suspended wire-bottom cages. These pans are slanted slightly in opposite directions under each tier of cages. Water overflows from a reservoir on top of the rack or is expressed from jets and flows across the pans, cascading from one level to the next and finally into the drain. Flush cycles are controlled electronically or by a gravity system. These racks are equipped with automatic watering, which may originate from the rack reservoir or from an external supply.

The front-to-back flush system has a water manifold under each tier of wire-bottom cages. This manifold is equipped with a series of small nozzles that spray water across the pan from the front of the cage to the back and into a trough or drain system. Flush cycles are controlled electronically and these racks are usually equipped with automatic watering.

Advantages

The major advantages of automatic flush caging listed by two or more respondents were, in order of frequency:

- labor or time savings;
- reduction of odor;
- elimination of bedding materials;
- reduction of disease-bearing particles;
- cleaner animals; and
- reduction of menial tasks (less drudgery).

Other advantages listed only once were: elimination of drop pans, reduction of human exposure to excreta, impressive appearance, ease of maintaining humidity level in rooms, less upkeep, and improved animal health.

Disadvantages

The major disadvantages of automatic flush caging listed by two or more respondents were, in order of frequency:

- high initial cost;
- requirement for special building design (plumbing, etc.);
- increased water usage;
- difficulty in observing morning feces; and
- need for technicians to understand the system to prevent malfunctions.

Disadvantages listed only once included: the unsuitability of the system for some special studies, necessity for some manual cleaning of flush pans, requirement for more setup time for racks, mineral buildup on flush pans, electrical maintenance, potential need for devices to eliminate "pounding" in water pipes, difficulty of moving heavy racks, buildup of feces in floor troughs, and elevations in room humidity.

Maintenance Costs

Most respondents using automatic-flush caging reported minimal maintenance costs. Repair and replacement of solenoids were cited most often. One facility routinely cleans out a water heater used for heating the flush water.

Cost Analyses

Five respondents sent in cost analyses of their automatic-flush caging systems.

Respondent A This time and function study was based on 21 automatic-flush rodent racks and 16 automatic-flush rabbit racks. The purpose was to compare the cost per animal to costs incurred with manual systems.

The study commenced with the designing of 2 forms--one for the automatic racks and one for the manual. The forms were printed on different-colored paper to minimize the chances of data being reported on the wrong sheet. There were spaces to record start and finish times for each of these functions, which were listed by species: feeding, watering, bedding, room maintenance, equipment maintenance, cage washing, animal care, and administration. Each day, each caretaker was

TABLE 1 Weekly Costs Per Animal

Species	Labor Cost per Animal ($)	Feed and Bedding Cost per Animal ($)	Total Cost per Animal ($)
Manual Racks			
Mice	0.097	0.019	0.116
Rats	0.409	0.098	0.507
Rabbits	2.567	0.250	2.817
Automatic Racks			
Mice	0.042	0.015	0.057
Rats	0.295	0.066	0.361
Rabbits	1.376	0.045	1.421

given sheets for the automatic and manual systems. A 2-day trial was initiated to work out problems in understanding the forms, etc. The final study involved having each caretaker record, not how many minutes he worked at a particular function, but what time he started and finished: That information allowed not only a good measure of the time utilized for each function, but it also gave the supervisor a chance to measure his staff's effectiveness. After the data were collected for 2 weeks, they were tabulated and the labor component was calculated by species. Averages of the inventories at the beginning and end of the 2-week period were used. Labor costs included salary and benefits. Material costs were collected by recording weekly quantities used by species (figures supplied by supervisors), applying current costs, and dividing by the average inventory. Following this effort, a separate team of accounting people observed the caretakers at work, conducted their own time study, and evaluated the material cost figures. Their conclusions were consistent with data provided by the study. The results of the study are set forth as Table 1.

Respondent A planned to make a revised study of animal-care rates because the mix of animals between automatic and manual caging had changed, and because efficiency in the total care system had increased.

Respondent B Respondent B performed cost analyses, but the data were not complete because the systems were not fully installed and the numbers of animals were not adequate to permit meaningful comparisons. One observation that emerged was:

> There was no question that fewer numbers of people were required to care for animals in automatic cages rather than conventional cages. There were also some unexpected observations, the most notable of which was the fact that rabbits turned out to be cheaper to care for than guinea pigs on an automated system.

Respondents C, D, and E Respondent C reported experiences and data very similar to those mentioned by Allen (1973). Cost savings of 21-45 percent for automatic watering alone and up to 63 percent for automatic waste-handling were cited. Before purchasing the institution's present system, Respondent D made a cost analysis of automatic versus manual systems. For the numbers of animals the facility would be housing and the numbers of technicians needed, installing an automatic system in institution D "would save money over the years." Respondent E stated that the "automatic flush reduced our per diem charges for rats and rabbits due to reduction of labor and bedding cost."

AUTOMATIC DRY-WASTE REMOVAL CAGING

Automatic dry-waste removal caging provides an alternative to automatic-flush caging. These systems rely on conveyor belts rather than flowing water to move excreta. There have been limited attempts at producing this caging commercially and some facilities have fabricated their own custom-made systems.

One of the first commercially offered systems is designed with paper laminate rolls under each tier of cages. The paper belts move automatically from a supply roll to a take-up roll. The excreta are sealed between successive layers of treated paper laminated on the take-up roll. A more recently marketed system has a modular conveyor assembly under each cage tier. The conveyor belt, constructed of fiber glass fabric impregnated with silicone, is motor driven—thus excreta are carried to one end of the conveyor and dumped into a disposable plastic bag. Automatic watering is a part of both of these systems.

Only one respondent reported experience with automatic dry-waste removal caging. Two main advantages were cited: savings of labor and cleaner room environment. Disadvantages included belt breakage and replacement. The respondent reported that maintenance costs varied with the complexity of the system. Costs primarily involved lubricating bearings and replacing belts. No cost analyses were performed.

CENTRAL VACUUMING SYSTEMS

Three respondents reported using central vacuuming systems.

Description

These systems generally employ a central vacuum power unit connected to the animal rooms by a series of manifolds. Flexible hoses equipped with special configuration nozzles are attached to the manifolds. The nozzles pick up bedding and excreta and evacuate it into a collection tank that is emptied periodically.

Advantages

One respondent was happy with the vacuum system and listed labor savings and maintenance of a clean environment as the major advantages. Another cited reduction of contamination in a barrier facility as a benefit.

Disadvantages

One of the respondents reported continual problems with clogging of the system, with a resulting effective operational time of 6 months over 6 years. An expenditure of a large sum of money has not corrected the problem. Another respondent said that an employee operated the vacuum improperly (he attempted to force too much impacted material into the nozzle at one time). The third respondent reported that high humidity during the summer months caused clogged lines, particularly in mouse rooms.

Cost Analysis

One respondent stated that manual cleaning required 42 manhours per week, whereas cleaning with the vacuum system required 18 manhours per week. These figures are for approximately 3,000 caged New Zealand white rabbits. The other two respondents did not perform cost analyses.

AUTOMATIC FEEDING SYSTEMS

Automatic feeding systems control the quantity of food given and the time of feeding. A commercially available system employs feed hoppers connected to a central electronic programming unit. The dry feed is discharged from the hopper into an attached bowl or tray at times and amounts specified by settings on the programming unit. This system is primarily used to feed large animals and fish.

Other systems that have been tried on a limited or experimental basis include rotating bins and overhead track units. Automatic pellet or biscuit dispensers in use for behavioral studies might be classified as automatic feeding systems, but they are usually not employed as the total food source during routine care of the animals.

None of the respondents reported experiences with automatic feeding systems.

DISCUSSION

The respondents represented a cross-section of academic and governmental institutions, industry, commercial breeders, and private laboratories, but by no means were they a majority of users of automated animal-care systems. They provided substantial information on experiences with automatic-watering and automatic-flush caging, but only three reported on central vacuuming systems and one on automatic dry-waste removal caging. None were using automatic feeding. These data may reflect the relative popularity of the various systems.

Savings of labor and time were nearly always cited as advantages of automation. However, only a very small number of respondents had actually conducted formal cost analyses. A definite need exists for this information, and it is hoped that this paper will stimulate further investigation and provide a framework for planning future economic studies. Since each facility is unique, cost studies should be custom-designed to meet individual features. Much information on cost analysis is available from the manufacturers of automated animal-care equipment. Several have conducted extensive studies and prepared case reports, but this research was excluded from this paper to reduce bias. Certainly the manufacturers should be consulted and their data considered before making a decision on purchase of equipment.

For many facilities, automated systems may be the answer for cost reduction and improvement of the animal environment, but they may be impractical in others. I have pointed out some of the factors that should be examined and weighed by prospective users of automated systems for animal care.

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Materials and Materials Function

ALBERT G. H. DIETZ

Materials of construction embrace such a vast and complex area that it is hopeless to try to do justice to it. Therefore, after briefly reviewing conventional materials, I have chosen to emphasize some relatively recent developments that may not be familiar to all practitioners. I shall also discuss those materials and attributes likely to be most applicable to animal facilities. Finally, proposals are made by which animal experts and building designers and producers may collaborate to evaluate and use materials more effectively than now appears to be the case.

CONVENTIONAL MATERIALS

Conventional materials, such as concrete, steel, wood, masonry, and glass, are generally familiar and will not be discussed in detail. A few points, however, are worth emphasizing.

Structural Distortion

It is important to have smooth, easy to clean, seamless, or crackless surfaces that do not provide lodging for bacteria or other contaminants. Structural engineers should be cautioned that enough stiffness and rigidity must be designed into the structure to avoid cracks in hard surfaces, such as concrete and plaster, or crack-prone walls and partitions, such as masonry, or the points where they join, such as floor-wall and wall-ceiling intersections, even under continuous and fluctuating loads. This requirement may call for more stiffness in the design than is usually necessary in buildings. Furthermore, materials, details, and construction methods should be chosen for their ability to minimize movements caused by shrinkage or changes in temperature and moisture content.

Concrete

Ordinary structural concrete has a tendency to form fine hairline cracks as it cures; in addition, it develops more fine cracks in tension under load. Prestressing, by putting the concrete in compression initially, can often minimize this effect and results in smaller-than-usual structural members. Prestressing, however, is not always feasible (Waddell, 1974). It is generally more readily accomplished with precast members in a shop than in the field, where the necessary jacks or other devices cannot be employed.

Steel

Designers have a variety of structural steels of different strengths available to them. If the design is for strength, certain steels can result in smaller, lighter members. Stronger steels are no stiffer than the others--thus, if the design is for stiffness, ordinary steels, at lowest cost, might as well be used.

The new weathering steels develop a tenacious coat of rust that protects the steel underneath from further normal atmospheric decay, thus eliminating the need for paint or other coating as preventives against these attacks. It does not necessarily protect the steel from other incursions, such as corrosion from chemicals. Until the rust is well established, it tends to wash off and stain other surfaces, such as concrete or

masonry. The rust may also rub off enough to be a liability if abrasion is important.

Wood

Modern wood adhesives are strong and completely waterproof. They can therefore provide laminated timbers of sizes and shapes impossible to obtain naturally. Often, laminated wood members are economically competitive with and may have properties not possessed by other structural materials. They are initially dry and will not shrink because they do not have to dry in place, but they can swell, shrink, and check (split caused by drying) with long-time changes in atmospheric humidity. Wood can be protected against decay, insect attack, and self-supporting combustion by a variety of treatments, many of which are suitable for indoor application (U.S. Forest Products Laboratory, 1974).

Plywood overcomes much, but not quite all, of the normal tendency of wood to shrink and swell. The surface veneers or facings, however, may develop fine cracks unless stable species of wood in very thin surface veneers are chosen. Surfacing layers of impregnated paper, pulp, or other materials can thwart this tendency and provide a good surface for paint and other coatings (U.S. Forest Products Laboratory, 1974).

Glass

Tempering puts the microscopic crack-weakened surface of glass in compression and the interior in tension. The compressive stresses must be overcome and turned into tension before the glass will break, resulting in greatly increased strength and resistance to blunt impact. When breakage does occur, the fragments are small and rounded instead of sharp and jagged. Glass must be cut, drilled, and otherwise fabricated to final size before tempering--subsequent cutting merely shatters it. Tempered glass has been known to fracture spontaneously if not properly tempered or if used under conditions that introduce extreme stress for which it was not designed (Watkins, 1969).

Radiation-absorbing, tinted, and coated glasses can greatly reduce the amount of sunlight transmitted, especially in the ultraviolet and infrared ranges, but the glass may become warm as it does so. The new photochromic glasses darken as the intensity of incident light increases and become lighter as the intensity diminishes. There is some time lag, especially upon relightening.

NEW MATERIALS

Plastics

Because many of the relatively new materials and combinations of materials or composites include or are based upon plastics and other polymers, it is worthwhile to look at these materials as a class. They constitute the fastest-growing group of building materials. Although the total quantity employed is small compared with the traditional materials, the number of different uses is probably as large as those of any other material (Dietz, 1969).

It must be emphasized that the word "plastics" embraces not just one, but some 20-30 often widely different molecular aggregations or polymers loosely recognized by the industry as being plastics. Interestingly, synthetic rubber, which meets all the criteria, is excluded, whereas the silicones, which depart in significant respects from the accepted criteria, are considered to be plastics. Cellophane, similar to many films accepted as plastics, is not. It is altered cellulose, a natural high polymer. Yet cellulose esters, such as cellulose acetate and nitrate, also radically altered cellulose, are plastics.

The two major classes are thermoplastic and thermosetting. Thermoplastics, consisting of separate long-chain molecules, become softer and more flexible as the temperature rises, which makes the chains more mobile, and become harder and even brittle as the temperature drops and mobility is diminished. In thermosetting plastics, chains or clusters of atoms are all cross-linked or interlinked chemically. Accordingly, there is little increase or decrease in mobility with temperature, and the plastics, once hardened or "cured," do not appreciably soften or harden as temperature changes. They may cure at room temperatures, but they are called thermosets because the earliest ones made required heat for curing, and many still do.

Although thousands of polymers are produced in the laboratories, not all have found use in building, but those that are have often made major inroads. Among the thermoplastics are polyvinyl chloride (PVC), polyethylene, the acrylics, nylon, cellulosics, polystyrene, fluorocarbons, and polycarbonate. Among the important thermosets are the unsaturated polyesters, epoxies, urethanes, phenolics, melamines, and silicones. Certain classes may be either thermoplastic or thermoset. Sometimes their most important uses are as copolymers, or as combinations of several different groups in the same molecule. Among them are soft flexible PVC-acetate film, and the tough acrylonitrile-butadiene-styrene (ABS). Plasticizers, stabilizers, antioxidants, fillers, pigments, and dyes alter the properties of plastics.

Major nonstructural uses of plastics include floor coverings, interior and exterior wall covering, natural and artificial lighting, piping and drains, fixtures, hardware, electrical parts including insulation, foams for thermal insulation, film for vapor barriers and many other uses, counter tops, furniture, waterproofing, sealants, and adhesives. Some are peculiarly useful for animal facilities because of their resistance to the contaminants found there; others are not. Each must be examined in light of the particular circumstances.

Coatings

Perhaps no other building materials have been as profoundly affected by the advent of the polymers

as have decorative and protective coatings (Banov, 1973). In many ways, this is the refinement of greatest importance to animal facilities because of the need for maintaining cleanliness and resistance to many different contaminants (Banov, 1973). The old, familiar words, "paint," "varnish," and "lacquer" are still used, but the constitutents and formulations have often changed radically. Now a wide range of vehicles or binders, resins, solvents, and other ingredients has greatly extended the versatility and the bewildering complexity of coatings.

Traditional drying oils have been joined and largely supplanted by alkyds and alkyd-oil combinations. Water-reducible acrylics, or latices, have gained great popularity. The alkyds and latices are used mainly for areas not subjected to more than mild wear and tear. For more severe to extremely hard wear, solvent-reduced acrylics, cellulosics, phenolics, melamine and urea, chlorinated rubber, chlorosulfonated polyethylene, epoxies, polyesters, vinyls, urethanes, and fluorocarbons provide a great range of materials with resistance to a correspondingly large spectrum of interior and exterior environments. Many combinations are possible. The epoxies may be combined with coal tar, polyamides, and polyimides, or formed into esters with drying-oil fatty acids. Silicones are commonly used as modifiers--e.g., they increase weather and chemical resistance of alkyds and polyesters. Baked silicone coatings resist high temperatures. Liquid silicones render absorptive masonry surfaces water-repellant.

All these synthetics are already in use in animal facilities and have helped to solve many problems. Epoxies and urethanes appear to be especially popular for hard and abrasion-resistant wall coatings; urethanes for floor surfaces and resistance to stains, alkalies, and acids; and vinyls for saltwater, oils, and chemicals. Probably the utmost in outdoor resistance is exhibited by the fluorocarbons: Lifetimes of 20-40 years are predicted.

Rubberlike membranes, tough, stretchable, and chemical-resistant, are based mainly on polyurethane, polysulfide, neoprene, and butyl rubbers. These are all binders. To them are added pigments and fillers of many kinds. The best binder can be defeated by overloading or underloading with pigment and filler or by choosing the wrong ones. Even more important is the preparation of the substrate, especially if metal is to be used in corrosive conditions. The substate must be free of dirt, grease, rust, moisture, or any other potentially deleterious substance. The best coating applied over an inadequately prepared substrate may fail just as fast as the cheapest.

Cost, of course, varies greatly. The most durable coatings are usually the most expensive. However, the cost of materials, even the most expensive, is a small fraction of the labor cost in preparing substrates and applying the coating. Even a cursory cost:benefit analysis shows the advantage of employing a coating that will last 10 years over one that will last 3 (Banov, 1973). Table 1 lists a number of coatings and their expected properties.

Composites

A major use for the polymeric materials, and a major trend in building materials generally, is in composites, combinations of materials whose combined behavior transcends that of the individual materials by themselves (Dietz, 1969). The three major classes are:

- particulate, which encompasses particles embedded in a matrix or binder;
- fibrous, which encompasses fibers embedded in a matrix or binder; and
- laminar, which encompasses layers of materials bonded together and possibly impregnated by a binder. In the laminar category, structural sandwiches are a special case.

Particulates Portland-cement concrete is the prime example of a particulate composite. Cast in place, precast, and as concrete block, it is the most-used material in building. Recently, however, polyesters have been substituted for portland cement to make *polyester concrete*, which is stronger in tension and shear than classic concrete, denser and less porous, and quickly curable, so it can often be used in a matter of hours after being placed. Thin building panels and wear-resistant flooring blocks are among the products. Large pothole repairs on heavily traveled roads have been made with concretes utilizing polyester-acrylic-methacrylic binders that cured and could be used within 2 hours. After several years of hard wear they are in good condition (Emery and Steinberg, 1976).

Veneer plasters made with polymeric additives can be applied in one or two thin coats, usually 0.15-0.30-cm thick, over substrates such as masonry and gypsum board. The plaster is ready for use 1-2 days after application.

Used in the manufacture of high-strength mortars, polymeric additives increase strength and adhesion to the point where masonry walls can sometimes be reduced in thickness by 50 percent. Prelaid panels can be hoisted into position instead of having to be laid in place.

When based on epoxies, topping for concrete floors can be thin, as opposed to the thick application needed for standard topping. It can be ground to form terrazzo. Patching can be performed in the same way. Cracks in concrete can be repaired with epoxies injected by hypodermic or other pressure devices.

Fibrous Composites Many fibers can be embedded in polymeric matrices, but the most common combination is glass fiber in unsaturated polyesters. The popular name is fiberglass, but reinforced plastic is a more accurate term (Broutman, 1969). Fiberglass shower stalls and bathtubs, some with integral surrounding walls, have become common. Lightness and toughness are among the advantages; a surface less resistant to scratching and staining than porcelain is a limitation.

Structural members, e.g. pultruded shapes, such as the familiar structural I and H shapes, circular tubes, channels, and angles, are strong but less stiff and more costly than steel. However, in corrosive surroundings they have a decided advantage in resisting rust and much of the chemical corrosion common in animal facilities. For tanks, the same advantages as for structural members apply. Shell structures in general are often advantageously made of these materials.

Flat and corrugated translucent sheets can be given varying percentages of light transmission. Sheets having as high transmission as glass are being used increasingly as solar energy collectors because of their light weight and resistance to breakage.

Laminates The number of combinations of layered materials of all kinds, put together in all the permutations possible, is virtually unlimited. A few of practical importance may be mentioned. The familiar counter and table tops, furniture facing, and door facings are made of high-pressure laminates. They are also shaped into utilitarian sheets and shapes for electrical and mechanical applications.

By definition, structural sandwiches consist of typically thin facings of hard, strong, stiff, dense materials over generally thick cores of softer, lighter, weaker materials. The combination is stiff and strong, yet lightweight, it often has good thermal insulating value, and it can be used for wall and partition panels, doors, furniture, and many other applications. A few examples follow (Rosato and Schwartz, 1969). Polyester concrete facings, each approximately 2.5-cm thick and reinforced with glass fiber, combined with cores of 2.5-cm polyurethane foam, provide complete outside wall and partition panels. The concrete is inherently dense and not porous, because nothing evaporates during curing. The surfaces can be molded to the desired texture and color. Such panels with integral gel-coat surfaces are claimed to be excellent enclosures for monkeys and similar animals. Highly translucent panels consist of thin (0.15-cm) facings of glass fiber, reinforced polyesters, or polyester-acrylic combinations, bonded to a grid of small aluminum extrusions. The resulting panels can bear moderate loads (one or two stories), and therefore provide a combination of structure, enclosure, and light transmission.

PERFORMANCE SPECIFICATIONS

Most specifications and codes for materials are prescriptive; they describe in some detail the characteristics of the materials and how they are to be applied. This format implies that the specifier knows that the materials, if they meet the specifications, will perform satisfactorily in service, that is, will meet the performance requirements. This is not necessarily the case! This, in turn, ususally implies that a given material has been used long enough and extensively enough to have proven itself in service. One consequence is that specifiers tend to avoid new materials, because they are unfamiliar and unproven in service, although they may be superior to those conventionally accepted.

To offset this tendency to exclude unfamiliar materials, now arriving in increasing numbers and conbinations, and to make sure that materials will perform adequately, specifiers are moving toward the adoption of performance specifications. The objective is not to describe the materials and how to use them, rather, it is to set forth the conditions that should be met and the performance required, and to assign responsibility for meeting those requirements to the materials supplier and the installer. The underlying concept of performance is a concern with what a building does, not how it is made.

The idea has much appeal, particularly in specialized cases such as animal facilities. It is assumed that the specifiers know what performance they want, but do not necessarily know all applicable materials and methods. Conversely, it is implied that the materials and methods specialists do know how to meet those requirements, once they know what they are.

Considering the ramifications of this approach, the Building Research Advisory Board of the National Academy of Sciences formulated the following fundamental elements of the performance concept (Building Research Advisory Board, 1965):

- Determinants: the owner's requirements, to be satisfied by the building.
- Functional requirements: features to be determined by the designer, needed to satisfy the determinants.
- Technical characteristics: properties of materials and equipment necessary to meet the functional requirements.
- Evaluative techniques: means of testing materials and equipment to see if they meet the technical characteristics.
- Standards and regulations: codes and standards developed on the basis of performance and requirements.
- Experience appraisal: gathering, analyzing, disseminating information on the functional, physical, and economic performance of buildings.

Determinants Do you, as owners and operators of such facilities, really know what it is you want, other than in broad generalities? Can you set forth those requirements in precise enough terms, without reference to specific materials and methods, to allow your designers, architects, and engineers to translate them into performance specifications? Can you be sure that even if the facilities are built according to your determinants, they will meet your requirements?

Functional Requirements Assuming the owners' determinants are good enough to assure obtaining the facilities they want, can the designers translate them into specific enough functional requirements, such as resistance to various at-

TABLE 1 Engineering Characteristics of Resin Families[a]

Binder Type	Typical Uses	Application		Cost[b]	Outdoor Life, yr	Color Stable, External	Gloss Retention, External	Stain Resistance	Weather Resistance	Abrasion and Impact Resistance	Flexibility
		Shop	Field								
Acrylics											
solvent reducible		yes	no	M	10	yes	good	fair	good	good	good
water reducible		yes	yes	M	5-10	yes	fair	fair	good	good	good
Alkyds	External primers and enamels	yes	yes	L-M	5	no	good to excellent	fair	fair	fair	fair to good
Cellulose acetate butyrate	Decorative high gloss finishes	yes	no	M	--	yes	good	fair	good	good	good
Chlorinated rubber	Corrosion-resistant paints; swimming pool coatings; protection of dissimilar metals	yes	yes	M	10	yes	fair	fair	good	good	good
Chlorosulfonated polyethylene	Paints for piping, tanks, valves	yes	yes	VH	15	yes	--	fair	excellent	fair to good	excellent
Epoxy polyamide	Moisture- and alkali-resistant coatings; non-decorative interior use	yes	yes	H-VH	15-20	no	poor	good	good to excellent	excellent	good
Fluorocarbons	High performance external coatings; industrial siding; curtain walls	yes	no	VH	20	yes	excellent	excellent	excellent	excellent	good
Melamine formaldehyde	Decorative high-gloss finishes	yes	no	M	10	yes	good	good	good	good	good
Phenol formaldehyde	Chemical- and moisture-resistant coatings	yes	yes	M	10	no	fair	fair	good to excellent	good to excellent	good
Polyesters	Piping; ceiling tile; cabinets; furniture	yes	yes	H	15	some versions	good to excellent	good to excellent	good to excellent	good	good to excellent
Vinyl	Bridges; offshore; chemical products	yes	yes	H	15	yes	good	fair	good to excellent	good	good to excellent
Silicone-modified polymers	High performance external coatings; industrial siding; curtain walls	yes	yes	H-VH	15-20	yes	good to excellent	good	good to excellent	good to excellent	good
Urethane (polyester-cured)	Heavy duty coatings for stain, chemical, abrasion, and corrosion resistance	yes	yes	VH	20	some versions	excellent	good to excellent	good to excellent	good to excellent	excellent

[a] Modified from Banov (1975).

[b] L = low; M = moderate; H = high; VH = very high.

tacking agents, suitable porosity, and flexibility in temperature range? In other words, if these functional requirements are met, will the owners' determinants be satisfied, and will the desired facilities result?

Technical Characteristics Once the designers establish functional requirements, can the materials specialists determine the properties of the materials, such as strength, durability, weight, hardness, and permeability, well enough to be sure that the functional requirements will be met?

Evaluative Techniques This is in many ways the crucial element. How can one test the chosen product, combination of materials, piece of equipment, or particular item thoroughly enough to be sure that it meets the functional requirements? This is not the same as a standard test of a material, because presumably no particular material has been specified. Instead, the potential expected performance of a product or service is being examined. No standard test may exist; new tests or combinations of tests may be required. Without such evaluative techniques, the foregoing steps are meaningless.

If these questions sound discouraging, they are not meant to be; they are meant to be realistic. The present prescriptive specifications frequently fall far short of meeting their objectives and, as indicated, often rule out promising new avenues. A pragmatic approach is a mixture of prescriptive and performance specifications, each used when it best meets the needs of the situation.

TESTING AND OBSERVATION

As has been implied, numerous and extensive measures have been developed for testing building materials under consideration. With its many committees, the American Society for Testing and Materials (ASTM) has been particularly active, and it has promulgated the standards and tests almost universally employed for building materials. As needed, new tests and standards are developed and existing ones are continually being reexamined and changed. By Society rules, committees must be made up of representatives from producers, consumers, and general interest groups, with no one element predominating. Every effort is made to avoid bias.

These tests, good and extensive as they are, are largely short-time laboratory tests, although long-time exposure tests, particularly in the case of coatings and corrosion, are employed. Short-time laboratory tests seldom can be relied upon to predict behavior over a long period, especially under the immense variety of conditions found in the field, unless extensive correlation with long-time field observations and tests has been observed. Unfortunately, well-organized and documented field observations are often lacking. We build our buildings, walk away, and do not go back to see how they are holding up.

The operating crews of buildings may know what their troubles are, but this knowledge seldom is communicated to the designers. Some large users of buildings, such as the General Services Administration and large corporate owners, undoubtedly have much information, but it does not appear to be generally available. Manufacturers also are apt to know about their failings as well as successes, but they are not likely to broadcast the failures.

The sixth element of the performance concept calls for experience appraisal. A well-organized, systematic program for observing actual behavior of materials in animal facilities for as long as possible could be of great help to designers in search of the right materials or the right performance specifications for the right places. The operators of such facilities know best what to look for. They could, of course, be assisted by architects and engineers as well as materials specialists. Such information, made available on a completely impartial technical basis, would be of great assistance in the search for new and improved materials to meet the requirements of users.

Although this kind of systematic study is badly needed for most buildings, it would be staggering if undertaken for all materials in all construction. For the smaller and more specialized area of animal facilities, although still a considerable undertaking, a survey ought to be more nearly manageable.

Returning to the formulators of tests and standards, such as the ASTM, it is curious that designers, builders, and code authorities make extensive use of its standards, but relatively few of them actually participate in setting them. The Society has recognized this discrepancy and would welcome greater involvement by these professionals to supplement and clarify the work now largely carried on by materials specialists, mainly chemists and chemical engineers. The field observation suggested above is one way in which the work of setting standards, and the materials specifications based upon them, could be strengthened. Carefully carried out systematic observations of actual field behavior are essential if the performance concept and the development of performance specifications (the fifth item of the performance concept) are to become practical and useful. Not until actual performance is known can specifications be written with confidence.

EVALUATION AND CERTIFICATION

When designers or operators of animal facilities are faced with an unfamiliar but attractive material, they need some way of evaluating it, especially if it is relatively new and does not have a long history of actual use. Some of the materials discussed above are in this category. Evaluation goes beyond just testing. It involves judgment as to the probable behavior of a material in use. Laboratory testing may

give strong indications, but laboratory tests and actual installations are not necessarily the same, particularly for an untried substance.

A procedure developed in Europe, called the *agrément* system after the French term, may offer assistance. When a new material or device is submitted to the Agrément board or agency for evaluation, an *ad hoc* group of specialists and experts familiar with the type of application involved is asked to review the item and the supporting evidence, and, if necessary, call for additional tests, field observations, and any other pertinent information. When the evidence is all in, the group considers it, evaluates it in the light of its collective experience, and decides on its applicability and under what conditions. A certificate is issued to the proponent, which he or she can use when seeing owners, designers, builders, code officials, and others involved in decisions respecting its use. The Agrément board reserves the right to review its decision from time to time in light of new experience.

The kind of systematic field observation of the behavior of materials and equipment suggested above would have striking advantages in any evaluation and certification process. The following recommendations are offered for the consideration of designers and operators of animal facilities:

- Organize a task force of operators and designers to draft and conduct a systematic and continuing survey of the actual performance of materials in animal facilities.
- Develop a set of performance requirements on the basis of the collective experience of the operators of animal facilities, and from the observations resulting from the survey recommended above.
- Organize a procedure whereby materials and equipment can be evaluated by panels of knowledgeable individuals, and their collective judgment of suitability set forth.
- The assistance of agencies such as ASTM and the American National Standards Institute should be employed to the fullest possible extent. In addition, the legal implications should be explored, e.g., what responsibility do individuals or a board assume if failures occur in spite of their best judgment?

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Discussion

PHELEN: I am Dr. Phelen from Smith, Kline, and French in Philadelphia. Dr. Dietz, one of the greatest materials problems that I have experienced as a colony manager is with laboratory animal floors. In my experience, most flooring has proved unsatisfactory including PVC, high-density concrete, regular concrete, rubber, vinyl, epoxy, quarry tile, and terrazzo. What kind of material will meet the specifications of a nonslippery surface that is also acid-resistant, alkali-resistant, nonporous, easy cleaning, resilient, and long lasting?

DIETZ: I do not know of any. There is a phenomenon familiar to designers, known as overspecification. Maybe you are specifying too much and you just cannot get it all. I will simply say that there is no such thing as the perfect material. Perhaps the compromise would be along the line of performance specifications, in which you attempt to set forth the most reasonable combination you can ask for and then leave it to the ingenuity of the materials people to find an answer.

PHELEN: I would like to address this question to one of the engineers. I have heard a great deal of interesting material presented today about energy recovery systems. At temperate times of the year, we often have the animal rooms getting too hot and the chilled water for cooling has been drained out of the pipes. Do any of these heat-conservation systems offer a solution to this?

SPIELVOGEL: No, but if you need chilled water year-round, there is no reason why you should not be able to have it. The question is, are you willing to pay for it?

LINDSEY: I am Russell Lindsey from the University of Alabama. Dr. Gorton, you reviewed the different energy-saving devices available, but you avoided putting any relative merit or value on them, or stating their advantages or disadvantages. I wonder if you might do that.

GORTON: You are going to get a series of negatives from us, I'm afraid. I would not like to do that because typically, to make the system more efficient, it is necessary to install a larger unit. Then there is a greater pressure drop across the system and more has to be spent on electrical energy to run the fan than is possible to recover from the system. So, without a detailed analysis of the particular situation and a particular climate, one can't be compared to another.

LINDSEY: I understand your point and I accept it, but I think some general statement should be made for each one of these devices. Also, some information should be available on which ones are the most fool-proof and fail-safe.

GORTON: The static ones, like the recuperator type or the contact heat exchangers, would be the most fool-proof, because nothing can go wrong with them other than getting clogged. So, you run into the complication of having to be particularly careful with the filters. Because of that sort of consideration, I find it very difficult to answer. Every time I

bring up a benefit that makes one superior to another, I can usually develop an argument that modifies that statement.

SPIELVOGEL: I have two points I would like to make. First, after 10 years of haggling and arguing, within a few weeks the industry is going to publish a standard method of rating heat-recovery devices that will at least enable comparisons from one to another. Also, in the preparation of ASHRAE Standard 90, heat recovery was probably one of the most controversial subjects. We had to consider whether to mandate heat recovery in buildings in which there was ventilation air. We found, after many meetings with the technical people, manufacturers, and trade groups, that situations existed in which the heat-recovery system used more energy than was consumed without one. We therefore decided to suggest that their use be considered but not mandated.

KOHLOSS: One point can be made about choosing one or another device. The run-around system, that is, two coils with the pipe and the connected pump, and the spray-desiccant system can be applied where the air inlet and the air outlet are widely separated. The others generally have to have a contiguous inlet and outlet. That is one variable that might be worth considering.

CASS: I am Jules Cass from the Veterans Administration. I want to compliment and support the suggestion that Dr. Dietz has made for establishing a mechanism for reviewing our animal study areas and construction renovations. The Veterans Administration has in its construction program a means for postoccupancy review of all its hospitals, including the research areas. We concluded our first review in the animal area about a year ago and we hope that this will become a regular practice. I would urge that this be done far more broadly.

GOLDSTEIN: I would like to take this opportunity to ask the audience for an expression as to how many would be interested in attempting to institutionalize Dr. Dietz' suggestion. Could I have a show of hands? Dr. Besch, have you noted that? We have seen a formal indication that a majority of this audience would be interested.

NELSON: I am Dr. Nelson from the University of South Florida in Tampa. I have made a great many observations about the architects and engineers here. They have done a fine job of getting the building to the point where it goes out to bid, but how do we get the building built?

GOLDSTEIN: I have learned Tender Loving Care in dealing with the medical fraternity of the bioscience community, very patient veterinarians, and animal facility managers. TLC is the only way to get something done in our society. That does not necessarily mean using kid gloves to handle people in the field; persons in the field must continuously be made aware that quality control is required for every system and every material that goes into a building. It is evident to all of us that standards of performance have declined in our society. Many times workmanship is not adequate, and materials are not delivered on time, which can compound the problems of quality control. To police a construction site requires a great deal of effort and costs money in terms of supervision time. However, the cost is minimal compared to the aggravation that the occupants of the facility will ever after have to bear. So, how is it done? With money, adequate coordination of manpower, and TLC.

MELBY: Ed Melby, Cornell. We are winding up a symposium to update one that was held 13 years ago. When this symposium is published it will be read by many of us who are actively engaged in the field as laboratory animal directors or directors of research facilities. I wonder how best to communicate the observations and findings that have been made here to the architects and the other people who are involved in facility construction. I think everyone here could tell a few horror stories of working with architects and others who will not read, will not listen, and feel that we should stay in our area of expertise and they should stay in theirs. How do we get our messages across? We are talking about very expensive facilities to construct and operate, whether they be private or public. It seems to me, as a taxpayer, or as someone who is going to be using the building, that these are important issues. Rather than just having them buried as minutes or memoranda or publications of symposia by the National Academy of Sciences, what can we do to get this into the trade media that people will read?

BESCH: I am a member of the American Society of Heating, Refrigerating and Air-Conditioning Engineers. About 18 months ago, I was chairman of a symposium that talked about the subject of environmental requirements for laboratory animals. Five people were involved--as I recall, two veterinarians, two engineers, and I. It was amazing to see the attendance of the meeting and the attentiveness of the audience, who were primarily engineers. The information presented became a part of ASHRAE literature. These handbook series, application volumes, systems volumes, and fundamentals volumes are sources of basic information for engineers and architects. That is at least one way to make information available.

GORTON: That very closely parallels what I was going to say. I think it would be reasonable to expect that you could get this information in a chapter in something like *ASHRAE Applications* volume, a book on every engineer's desk. In essence, it is a manual of recommendations, observations, and standard practices that an engineer incorporates into his designs. So, if we assembled the material properly, I am almost sure we could get a chapter inserted in the ASHRAE guide, which

very specifically then relays the message to the engineers involved in the design of the facility.

SPIELVOGEL: I think I can say the same for the *Illuminating Engineering Society Handbook*.

MORELAND: I am Dr. Moreland from the University of Florida. Professor Dietz, many facilities in this country house dogs indoors. Almost all of them use some sort of concrete surfaces for walls and ceilings. You used an interesting term, "acoustical attenuation," in your presentation. Can you suggest how to attenuate the acoustics in a room full of dogs where the room is constructed of concrete?

DIETZ: That is a real problem and I have no solution for it.

GELLER: I am Dr. Geller from Albert Einstein College of Medicine in New York. I want to ask Dr. Hickey if the temperature of the water used in automatic flushing cages, presumably cold water, affects the temperature in the environment of the animal and in the room?

HICKEY: That depends on the particular institution and the incoming water supply. In our own case, the incoming water seems to be of an adequate temperature year-round. The room environment isn't affected. Now, I must hasten to add that you should be most careful to check coldness at cage level, rather than simply in the room. Sensors placed within the cage environment during flush cycles would determine adequately if your incoming water is too cold. If the temperature does fluctuate markedly, then you might have to consider auxiliary heating of the water supply. In some areas, that is not necessary. Other areas have to do it routinely. It depends on your own situation.

KOHLOSS: Dr. Gorton and his colleagues wrote a paper on the effect of cold water on the animal-room environment.

GELLER: We are talking about labor-saving devices for reducing the expense of maintaining an animal facility, but we are also taking away employment from animal-care people. Sometimes it bothers me when I think about eliminating jobs this way.

HICKEY: Just to give you a personal example, when automated systems were installed in our facility, immediately technician-level positions opened up to replace some animal caretaker jobs. We gained animal observers, animal technicians, and we did away with a portion of the animal caretaker drudgery. So, that can be a factor, too.

GOLDSTEIN: I would like to make a philosophical comment. At some point, it may be necessary to discover if automated systems are consuming too much energy. That aspect will have to be taken into account at some point in budgetary considerations.

PHELEN: I would like to respond to Dr. Moreland's question concerning acoustical materials with some experimental work we have done. Some years ago, the 3M Company came out with a material that resembled a fiberglass lining coated with a very thin, probably mylar, film. It was supposed to be a very fine acoustical material. We theorized that hanging this material from the ceiling would at least stop the bouncing effects of noise in the dog rooms.

We hung these panels approxiamtely 60 cm apart, suspended vertially over the entire length of the room. Panels were also hung longitudinally. We did reduce the decibel level of the sound that was producing an echo effect, but the noise dosimeters that our animal technicians were wearing showed that we were unable to stop direct, line-of-sight transmission of sound. We ended up with very high cumulative noise levels in the dog rooms. Another reason for using this particular material was that it was autoclavable, and we were concerned that any acoustical material hanging in the animal room would act as a fomite. This material could be unhooked from the ceiling and sent through a regular cage washer at 82.2°C. However, it did not cut down noise in any appreciable way.

V

Summary and Challenges for the Future

Summary and Challenges for the Future

THOMAS B. CLARKSON

With more than a decade elapsing since the general subject of laboratory animal housing was considered in a national forum, it seems appropriate that engineers, architects, and laboratory animal scientists have gathered together for these 2 days to consider recent advancement in this area. This symposium is divided into 4 topic areas and each topic is assigned a portion of the program. These published proceedings provide detailed information about each of the topics. I will make no effort to review the subjects in a comprehensive way; rather, certain general observations that seem important are restated and special attention is paid to challenges for the future.

SESSION I

The first session dealt with animal facility management and design in terms of performance, and it was intended to report up-to-date information acquired since the symposium on the same subject held in 1963. From the data presented, it seemed clear that at least half of the animal facilities surveyed had not met the expectations for which they were designed, and moderate-to-extensive renovation had been necessary for their continued employment.

Several generalizations can be made that relate to inadequacies of facilities. The most general deficiency seems to have been the lack of involvement of an experienced laboratory animal medicine specialist in developing the plan. As a result, veterinary support space (diagnostic laboratories, recovery rooms, X-ray facilities) has been insufficiently allotted, and space assigned to laboratory animal housing has been diverted to those functions. Other problems have been the lack of anticipating change in facilities, difficulty in expanding, faulty or limited performance of construction material and equipment, and, finally, mechanical equipment that is too complicated to be maintained by most university maintenance persons.

Observations were reported that related to the question "Does management of facilities complement the design of facilities?" A major question was whether clean-dirty corridor systems were used once they were built. Based on a survey of 61 institutions, most facilities were not using them as they were intended. A double-corridor system seemed to work less well in medical school situations than in industrial or breeding laboratories. This discussion did focus on a challenge for the future--the need for experts in animal facility design and management to emerge from the disciplines of animal laboratory science, architecture, and engineering if the designs of the future are to complement management better.

Controversy continues about the suitability of centralized, as compared to dispersed, animal facilities for particular institutions, perhaps because the issues concern such powerful intangibles as academic politics, local tradition, the investigator's desire for control clashing with the administration's desire for control, and the scientific needs of programs and their relationship to anticipated scientific productivity. It is helpful that an estimate was given for the minimum size of an animal facility to be considered functionally effective as a satellite facility. That minimum size is estimated at 450 m^2.

In the discussion of centralized versus dispersed facilities, a strong point has been made. If dispersed facilities are used, nevertheless,

a core facility must exist to provide a home base for the specialist in laboratory animal medicine, other kinds of laboratory animal scientists, and support laboratories for the whole university. Experience has shown that a centralized personnel program works better and that coordination of that activity should be based in the core resource.

The architectural contribution to the first session concerned the organization of planning information and the relationship of the derived plan to architectural theory. A useful point was made about the need for proper organization of the masses of information that are usually provided to animal-facility planners. Particularly important has been the need for informed setting of priorities as facility designs progress. The need for flexibility in animal facilities was reinforced and an architectural concept of opened versus closed geometry was presented.

The last discussion of the session has brought into focus two kinds of future needs. The first involves a means of recognition or accreditation of individuals with established expertise in animal facility design among architects, engineers, and laboratory animal scientists. The second concerns the need for performance evaluations of equipment and construction materials. Such evaluations should be made by a team of experts on a continuing basis and the results published in the literature.

SESSION II

The second portion of the symposium concerned the laboratory animal's environment. Early in the session, the importance of physical, chemical, and microbial factors affecting biological responsiveness in animals was reviewed. A unifying concept was presented, defining biological response as the sum total of environmental and genetic factors, and examples of variations observed in biological responsiveness associated with environmental factors were documented.

Social behavior of animals in a laboratory environment was discussed, particularly the effect on experimental variation of physiological events leading to behavior fluctuations. A new view was given of laboratory animal crowding--crowding is a function of the number of animals and not the square centimeters of space available to each animal. Another new point of view concerned aggression, which can probably be controlled genetically.

The second session contained a detailed discussion of primary and secondary enclosures, using those terms to describe the room and the cages within the room. This discussion also focused on a key future need: that standards for cages include performance data on heat and air exchange. Relatedly, another presentation of the second session concerned integrating objectives in the engineering design.

The concept of acknowledging human needs in animal facility design has provided me with many fresh and useful thoughts about animal facilities. The personal requirements of workers are probably the most widely overlooked and under-evaluated of all the aspects of the animal facility. We spend up to 90 percent of our waking hours at our work place. Naturally, the facility design should take into account not only our professional and technical needs, but our requirements as human primates as well. Over the years I have observed, without realizing the meaning, many things that workers do in attempts to stake out their territoty, create privacy, and to establish personalized surroundings as they go about their tasks. In the future, I know I shall seek the advice of an environmental psychologist if I am concerned with the design of an animal facility.

SESSION III

Containment of hazardous agents is an area that has probably gained in prominence and sophistication more since the 1963 symposium than any of the other subjects presented. The whole topic is probably too broad, complicated, and important to be merely a fraction of the symposium. A symposium on biohazard containment alone is needed now; any such endeavor should provide a comprehensive education and orientation for all people involved in managing a laboratory animal facility.

The opening of the session has reviewed the broad question of the need for hazard containment. We may need to seek informed consent from employees before exposing them to a potentially hazardous environment. Correspondingly, we have to recognize the psychosocial impact of working with biohazards.

Participants in Session III have presented much excellent information on the containment of infectious diseases. The hazards of working with infectious diseases can be reduced by thorough training of personnel, proper barriers, adequate animal restraint, and satisfactory disinfection. A need exists to reexamine classification of hazard. Presently, one must combine the standards provided by the Center for Disease Control and the National Cancer Institute to arrive at a classification. Cubicles in an animal room will have to be designed with ultraviolet light and unidirectional airflow.

Papers in this session also have dealt specifically with chemicals and toxins in laboratory animal facilities. Because more than 100,000 substances are known to be toxic, it is clear that administrators must have established practices for storing and handling hazardous chemicals. It is inadequate to tell workers simply to be careful. For safety's sake, all procedures should be written down and available to everyone in the facility. The often overlooked fact that many anesthetic agents are both chronically toxic and potentially explosive reminds the facility planner of the necessity for taking special precaution in designing surgical facilities. It is well to be reminded also that the two places most likely to be contaminated are the cage washer and the incinerator.

That commercially obtained animal feed is a source of chemical contamination in an animal facility is a somewhat new notion. Surprisingly, with even a limited study, investigators are able to detect toxin concentrations of vitamin D, lead, and arsenic in animal feeds. A challenge for the future is to establish a mechanism for assuring proper quality control of animal diets. As research becomes more sophisticated, it is no longer acceptable to feed animals potentially toxic diets of unknown composition.

Monitoring the quality of containment within a facility is also a subject under discussion. One way to check the spread of contamination is to add fluorescein to animal diets and then determine its spread through the facility by identification through ultraviolet light. This is an inexpensive method for testing a staff's ability to keep hazardous substances contained.

Another topic under study is aspects of design as they pertain to hazard containment. One of the first design criteria is to relate levels of risk to the facility plan. For example, many low-risk operations can be carried out using open benches. Those of moderate risk require a primary barrier, and agents of high risk require isolation and a primary barrier. Mechanical control of airflow for biohazard research has been reviewed. Future difficulties may involve acquiring the precision necessary for measuring the concentration of certain carcinogens in the exhausted air when the law specifies that less than 1 part per billion is the maximum allowable concentration.

Several papers recorded historical accounts of illness among laboratory workers; some of these infections could have been prevented by proper engineering. An analysis of five microepidemics showed that the engineering feature that would have been most valuable in their prevention was control of access to contaminated areas. Directional airflow and solid waste sterilization were also of major importance. Although preventive engineering features would have been very helpful in preventing laboratory-acquired illnesses, perhaps of equal importance would be training and discipline in laboratory personnel. Accordingly, no discussion of biohazard containment would have been complete without a consideration of the need for training and surveillance. Education and checkups would be necessary for both the hard-to-teach principal investigator and the easier-to-teach lower professional.

Items important for future can be summarized as follows:

- Need for more research on laboratory safety.
- More emphasis on safety and biohazard containment during training of specialists in laboratory animal medicine.
- A unified classification of hazard.
- A more extensive coverage of the problems of biohazard containment in the next revision of the *Guide for the Care and Use of Laboratory Animals*.
- Increased importance of adequate biohazard containment as a requirement in the accreditation process for laboratory animal facilities.
- A system for controlling the composition of laboratory animal diets.

SESSION IV

The final session has focused on cost-effectiveness in the design and operation of laboratory animal facilities. Some of the major considerations in this session have to do with the cost impact of arbitrary requests for such features as range of temperature and humidity control and numbers of air changes per hour. It is often stated, for example, that an animal facility must be able to vary temperature between 18.3 and 29.4°C. The basis for this range is uncertain, and the traditional specifications may be costly. Similar traditions exist for humidity control. It is often said that humidity must not go below 35 percent or over 50 percent. There seems to be no good reason that it could not go as high as 60 percent, and less specificity would be more economical.

The discussions on energy sources, the cost of energy, and the specific air changes per hour are very timely. It is usually said that 12-16 air changes per hour is good practice. Having been a party to such peer judgment, it is clear to me that not enough thought has been given to varying air changes according to the quantities and kinds of animals contained within a room, the sanitation practices, and the relationship between air changes within the animal room and within the cages in the room. In the future, financial and ethical imperatives for energy conservation may dictate that many of these "rules of thumb" be reevaluated.

To complete the session on cost-effectiveness, we have been reminded of the importance of considering automated systems for animal care, particularly because of ever-increasing labor costs. Construction materials can also contribute to savings in that they determine the life of an animal facility as a useable resource and the prevalence of "tight cracks" and "strong joints" in the facility.

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