Microenvironment in Ventilated Animal Cages with Differing Ventilation Rates, Mice Populations, and Frequency of Bedding Changes

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Abstract _ The purpose of the study was to assess the microenvironment in separately ventilated mouse cages at differing ventilation rates, mice populations, and frequency of bedding changes. We monitored intracage temperature, relative humidity, and concentrations of ammonia and carbon dioxide during 3 experiments. First, the effect of ventilation rate on the microenvironment of cages housing adult male mice was evaluated at 30, 40, 60, 80 and 100 air changes/h. For all ventilation rates tested, ammonia concentration was less than 3 ppm, carbon dioxide concentration ranged from 840 to 3,300 ppm, relative humidity ranged from 42 to 65%, and temperature ranged from 23.2 to 25.3°C. Second, we monitored cage microenvironment continually in experiments during which changing of bedding was delayed. Male mice were used in the experiment, and cages were ventilated (60 air changes/h). Cages were allowed to accumulate soiled material for 26 days, during which time ammonia concentration and relative humidity did not exceed 10 ppm and 45%, respectively. Third, we tested ventilation rate and frequency of bedding changes in ventilated cages containing breeding trios (2 females, 1 male) and their pups. Ammonia concentrations remained at 25 ppm or less for 30, 60, and 100 air changes/h when bedding was changed weekly and for 100 air changes/h when bedding was changed every 2 weeks. We concluded that 30 air changes/h was sufficient to maintain a healthful microenvironment in cages that were housing adult male mice in which bedding was changed weekly. When frequency of bedding changes was reduced to every 2 weeks, 60 air changes/h was sufficient for cages housing adult males, but 100 air changes/h was necessary for cages housing breeding trios and pups.

Caging systems are an important element in experiments, because the type of cage has a major effect on the degree to which conditions in the cage (microenvironment) reflect those of the animal room (macroenvironment) (1–5). Rodents used for research historically have been housed in cages with wire-mesh tops that do not provide a barrier to room air. Therefore, efforts to control the cage environment focused on regulating conditions in the animal room. With the development of filter tops to reduce airborne transmission of disease among animals, the degree to which microenvironmental conditions reflected those in the macroenvironment was also reduced. Use of tops on cages that do not have forced ventilation or use of unventilated micro-isolator cages decreases cage ventilation and produces major differences between micro- and macroenvironmental conditions (3–8). Most importantly, ammonia can accumulate in unventilated cages, and temperature and relative humidity are also higher in unventilated cages than in animal rooms (3, 6, 9–11).

Cages with forced-air ventilation or pressurized individually ventilated (PIV) cages were developed at The Jackson Laboratory, where they have been used since 1963 (12). Air is forced directly into each PIV cage, whereas ventilation of unventilated cages depends on ventilation of the animal room. Benefits of PIV systems, compared with unventilated cages of any type, include their ability to maintain low ammonia concentrations and a relatively dry environment in a cage (3, 13–15), reduce the spread of infectious diseases within a rodent colony (8, 12, 16), enable investigators to increase cage density, and reduce allergen concentrations and odors in the room. Disadvantages include high costs associated with various aspects of PIV systems, including purchase of the system, maintenance and replacement of filters, and electricity for operating the system (2, 8).

The role of PIV systems in retarding ammonia accumulation has particular importance for maintaining healthy rodent colonies. The primary source of ammonia in rodent cages is the conversion of urea to ammonia by urease. Urease is endogenous to some types of bedding (17) and is produced by urease-positive bacteria (6, 18–20). High humidity can enhance proliferation of bacteria and ammonia production in rodent cages. Ammonia accumulation is slowed when a dry environment is produced by efficient ventilation of PIV cages, and it may be possible to reduce frequency of bedding changes while maintaining low (harmless) ammonia concentrations. In a previous study of the effect of room-ventilation rate on microenvironment of unventilated mouse cages (21), we found that harmless concentrations of ammonia and relative humidity were maintained in cages at a room-ventilation rate of 10 air changes/h and an intracage-ventilation rate of only 8.7 air changes/h. Because higher air-flow rates are maintained in PIV cages than in unventilated cages, we investigated ventilation rate and frequency of bedding changes required to maintain healthful conditions in PIV cages. Finding the optimum ventilation rate and frequency for bedding changes could reduce costs for labor, cage sanitizing, bedding materials, and sewer fees.

The Guide for the Care and Use of Laboratory Animals historically recommended 1 to 3 bedding changes/wk for cages housing small rodents (22). The 1996 Guide revision recommended that frequency of bedding changes be determined on the basis of the professional judgment of animal care personnel and investigators, considering such factors as ammonia concentration, cage appearance, bedding condition, and animal density and biomass (23). Although the microenvironment of ventilated cages maintained at 23 to 250 air changes/h has been described (3, 9, 13, 14, 24), the degree of ventilation actually required to maintain healthful conditions in PIV cages has not been determined. The Jackson Laboratory currently operates its PIV system at approximately 60 air changes/h, but the microenvironment has not been quantified at other ventilation rates. The goals of the study reported here were to characterize the microenvironment of PIV cages for a range of ventilation rates and for differing cage populations and to evaluate the effect of delayed changing of bedding for specific ventilation rates.

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Materials and Methods

Animals: Mice used in the initial 3 experiments were C57BL/6J males, 9 to 11 weeks old, weighing 31.1 6 0.8 g and housed in groups of 4 mice/group. For experiment 4, C57BL/6J mice were mated in trio groups (2 females, 1 male) at 4 weeks of age. Pups in experiment 4 were removed from cages each Monday, Wednesday, and Friday at 28 6 3 days of age. To control cage density, we removed pups so that none of the cages contained more than 12 pups that were 14 days or more old, but we allowed cages to contain an unrestricted number of pups that were less than 14 days old. Mice in all experiments were housed in duplex polycarbonate cages (31 x 31 x 14 cm) divided into 2 pens and each was covered by a wire-rod metal top that held feed pellets and a water bottle. Cages were covered by a snap-on Lexon filter top (Reemay 2033, Thoren Caging Systems Inc., Hazleton, PA). Mice were allowed ad libitum access to bottled acidified water (pH 2.8 to 3.1) and pelleted feed. During the initial 3 experiments, mice were fed a formulated diet (Old Guilford 911 diet, Emory Morse Co., Guilford, CT), whereas those in experiment 4 were fed another diet (D11 diet, Purina Mills Inc., Richmond, IN). Autoclaved white pine shavings (Crobb Box Co., Ellsworth, ME) were used as bedding for all experiments. A 12-h light:12-h dark cycle was used. Analysis of an aerobic bacteriologic culture of representative fecal samples revealed urease-producing Proteus mirabilis was evident in feces of mice.

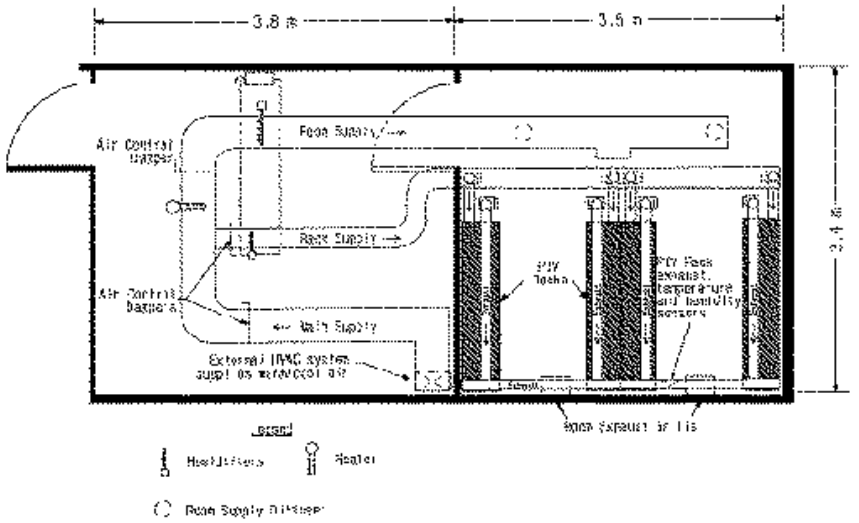

FIG. 1. Floor plan of mouse room (Room A). Air supply to the racks was heated, cooled, and humidified independently of air supply to the room. Sensors located in the exhaust duct of the pressurized individually ventilated (PIV) racks controlled the temperature of air supplied to the building through the main duct, which passed through high-efficiency particulate air (HEPA) filters. A second heater, located in the room supply duct, was used to maintain appropriate room temperature. Air control dampers and blast gates were used to regulate the flow of air into the room and the racks. The Heating Ventilation Air-Conditioning (HVAC) system was external to the room.

Carbon dioxide, ammonia, temperature, and water vapor: For each experiment, we concurrently monitored 3 occupied test cages, an empty control cage, and the animal room. Monitoring for experiments 2 and 3 was performed during 1-h periods beginning at 8 a.m., 11 a.m., 2 p.m., and 5 p.m. During experiment 4, monitoring frequency was decreased to 3 measurements obtained between 1 p.m. and 5 p.m. On each day that monitoring was performed, a mean was calculated from the 3 or 4 samples obtained for each specific cage. An unoccupied cage containing bedding, feed pellets, and water served as the control cage for each experiment.

Microenvironment variables were monitored via 2 fittings inserted near the bottom of each cage front. One fitting held a temperature probe that extended a distance of 13 cm into the cage, and the other held a gas-sampling line. All variables were measured, using a multipoint gas analyzer (model 1302, Brüel and Kjær, Nærum, Denmark). Measurement accuracy was 2.5% for gases and water vapor and 6 0.2°C for temperature. Water vapor and temperature were used to determine relative humidity. Monitoring of macroenvironment variables in each room was performed at a point approximately 1.7 m above the floor at a central location in the aisle between PIV racks. With the exception of the control cage, all cages in a rack contained mice.

Animal rooms and caging systems: Mice in all experiments were housed in PIV cages (Maxi-Miser® PIV; Thoren Caging Systems, Inc., Hazleton, PA) mounted on fixed or mobile racks. We carried out the initial 3 experiments in a small test room (Room A) described elsewhere (21, 25) that was modified to accommodate fixed-ventilated caging (Figure 1). Four fixed racks in Room A were supplied with outdoor air that was passed through a high-efficiency particulate air (HEPA) filter, using the building's central ventilation system. Exhaust air was directed outside the building. Each rack was 9 shelves tall by 5 cages wide, providing a room capacity of 180 cages. Outdoor air that had passed through a HEPA-filter was supplied to Room A at a rate of 10 6 1 air changes/h with a relative humidity of 45 6 5% and a temperature of 21 6 2°C.

Because of the additional space required for maintaining breeding colonies, experiment 4 was carried out in a large (5 X 12.8 m) working room (Room B). Room B contained 19 nonventilated animal racks and 3 mobile ventilated racks, and the experiment was performed by using the mobile racks. Mobile racks were 8 shelves tall by 4 cages wide, and total maximum cage density of Room B was 852 cages with a typical working density of 615 6 40 cages. Air that had passed through a HEPA-filter was supplied to Room B (15 6 1 air changes/h, relative humidity of 45 6 5%, and temperature of 22 6 2°C). Room air was supplied to the cages by a variable-speed blower mounted above each rack.

Procedures: Experiments were performed in an AAALAC-accredited animal facility and were approved by the Institutional Animal Care and Use Committee.

Experiment 1; variation of cage ventilation within a rack and influence of mice on cage ventilation: Before assessing effects of ventilation on the microenvironment, we determined whether all cages within a rack received equal amounts of ventilation. We tested ventilation rate at 9 cage positions on the rack in Room A on 3 days. Test cages were located in shelves 1, 5, and 9 of the 2 end columns and the center column. We determined the influence of mice on cage ventilation by comparing ventilation rate in cages with and without mice. We chose 4 cage positions for the test, and each cage was tested (with and without mice) 3 times each.

Experiment 2; effect of cage ventilation: Effect of ventilation rate on microenvironment conditions in cages containing male mice was evaluated for 30, 40, 60, 80, and 100 air changes/h. Each ventilation rate was maintained for 1 week. Microenvironment was monitored on the sixth day at the time of maximum accumulation of animal waste. Bedding was changed on the seventh day, and ventilation rate was increased. The cage-ventilation system in this experiment was fixed, and cages received only fresh outdoor air. Microenvironment variables measured were temperature, relative humidity, and concentrations of ammonia and carbon dioxide.

Experiment 3; effect of reduced cage-changing frequency: We evalu-

ated the effect of frequency of bedding changes on the microenvironment. Bedding in cages containing male mice and ventilated at 60 air changes/h was not changed for 26 days. The same microenvironment variables measured in experiment 2 were monitored on days 7, 14, 21, and 26. In addition, ammonia concentrations were monitored each Monday through Friday from day 14 through 26. The cage-ventilation system was the same as that used during experiment 1.

Experiment 4; effect of ventilation and decreased frequency of bedding changes for breeding colonies: This experiment was a combination of experiments 2 and 3, but used breeding adults with their unweaned pups instead of adult male mice. Microenvironment variables measured were the same as for experiments 2 and 3. Cages contained breeding trios (2 females, 1 male) and their litters. Mean number of pups in all experimental conditions ranged from 6.5 to 12 pups/cage at the time of monitoring. There were instances when cages did not contain pups at the time of monitoring; these data points were omitted from the analysis.

Three mobile racks ventilated at 30, 60, or 100 air changes/h were used. Within each rack there were three groups of cages, changed once every 7, 14, or 21 days. Combinations of 3 ventilation rates (30, 60, and 100 air changes/h) and 3 frequencies for bedding changes (7, 14, and 21 days) produced 9 experimental conditions, and data were collected from 3 cages for each of the conditions. We monitored all cages once a week for 9 weeks on the day prior to bedding changes. Cage air was drawn from and exhausted to the room through a HEPA filter.

To determine the biomass in each cage, the number of adults, number of pups, and pup age were recorded at the time of monitoring. A series of weights of the mice was collected to establish standardized weights for adults and pups. Weight for adult breeders was derived from the mean weight of 10 females and 10 males 148 days old. Pup weights were derived by weighing 3 litters at 3-day intervals from 1 to 28 days old. Standardized weights were multiplied by the number of pups at each age to determine biomass of the pups at the time of monitoring. Pup biomass was added to the weight of the breeders to obtain biomass of each cage.

Cage air-change rates: Ventilation rates were set by adjusting dampers in the supply and exhaust ducts of the racks. A ventilation rate was considered stable once the mean air-change rate at 3 cage positions, calculated 3 times for each position, was within 3 air changes/h of the targeted rate. The 3 cage positions used to measure a ventilation condition were in shelves 1, 5, and 9 of the third column of a fixed rack or in shelves 1, 4, and 8 of the second column of a mobile rack. Cage air-change rates were determined by using a multipoint-tracer gas doser/air sampler (model 1302, Brüel and Kjær, Nærum, Denmark) and photo acoustic infra-red gas analyzer (model 1303, Brüel and Kjær, Nærum, Denmark). The tracer gas was a mixture of 1% sulfur hexaflouride (SF6), and 99% nitrogen. Air-change rate was determined with the constant-concentration method in a 10 to 30 min test, using the following equation: $N(t) = F(t)/V(C)$, where N = air-exchange rate (cage volume/h), t = time (h), F = rate of introduction of tracer gas into the cage, (m^3/h), V = volume of air in the cage (m^3), and C = concentration of tracer gas in cage air (m^3/m^3). For this method, cage-ventilation rate is directly proportional to tracer gas emission rate required to maintain a constant concentration of tracer gas.

Determining cage ventilation: We attempted to maximize the accuracy of our tracer gas measurements. We confined dosing and collection of samples for measuring tracer gas concentrations to the right-side pen of the duplex cage. Volume of this pen was approximately 5,700 ml. Samples recovered were a fixed amount of 1,000 ml/min, because there was not an adjustable stroke volume on the sample collection pump built into the tracer gas doser/analyzer. In our previous study of unventilated cages (21), we determined that removal of a 1000-ml sample represented a substantial fraction of available air; therefore, measurements of air changes/h had to be corrected for this effect. In the study reported here, however, removal of a 1000-ml sample did not have an effect, probably because 1000 ml represented an insubstantial fraction of the high volume of air supplied.

To determine the effect of sample collection flow rate, we used a sulfur hexafluoride detector/chromatograph (ITI model 505, Ion Track Instruments, Wilmington, MA), which allowed sample collection at low and high flow rates. Four tests were conducted at each of the following flow rates: 140, 300, and 1,000 ml/min. Measurement of mean number of air changes/h at these rates were 26.0 6 0.2, 26.3 6 2.2, and 25.8 6 0.3. These means were not significantly different, as determined by use of an ANOVA, indicating that sample collection at 1000 ml/min did not induce false air-change rates. Static pressure of cages was positive during all ventilation testing.

Obtaining accurate measurements with tracer gas requires even mixing of tracer gas and cage air, but we observed uneven mixing in the cages. Two steps were used to minimize the effect of poor mixing. First, we collected samples of cage air from multiple intracage locations. Samples were obtained via 20 small holes drilled along the length of an 8-in Teflon line with an inside diameter of 0.19 in. The line was located on the bottom of the grain hopper, immediately above the zone occupied by the mice. Second, we placed a small (4 cm^2) fan in the front of the cage near the cage air-supply inlet to promote initial and thorough mixing of tracer gas and cage air. Wire screening (0.5-cm mesh) was placed around the fan for tests conducted when mice were in the cage. The fan was run continuously at its lowest setting throughout the tracer gas dosing/sample collection period.

To evaluate the fan's effect on ventilation rates, we compared ventilation rates calculated with the fan off and with it on. In 6 tests conducted with the fan off, values ranged from 21.9 to 34.0 air changes/h (mean, 26.4 6 2.2 air changes/h). In 4 tests conducted with the fan on, values ranged from 25.8 to 29.2 air changes/h (mean, 26.8 6 0.3 air changes/h). We concluded that use of a fan reduced test-to-test variability, but did not have an overall influence on ventilation-rate calculations. A previous study (26) in another laboratory also documented uneven mixing in the caging system that was used in the study reported here, confirming that these precautions were necessary.

Cage static pressure: An electronic micromanometer (Airdata Multimeter ADM-870, Shortridge Instruments, Inc., Scottsdale, AZ) was used to determine rack and cage static pressure. In Room A, supply pressure was measured in 3 occupied cages for each ventilation rate and was +0.1 Pa for 30, 40 and 60 air changes/h; +0.2 Pa for 80 air changes/h; and +0.7 Pa for 100 air changes/h. In Room B, cage pressure was measured in 3 unoccupied cages and was +0.1, +0.1, and +0.2 Pa for 30, 60, and 100 air changes/h, respectively. The slightly increased cage pressure for 100 air changes/h in Room A reflected the fact that Room A had 4 fixed racks, all operated by use of a central air supply and exhaust system. Racks in Room B were mobile, each rack having it's own motor and filter system, allowing a closer regulation of supply and exhaust air.

Statistical analysis: Values were reported as mean 6 SEM. An ANOVA followed by Tukey HSD post-hoc tests of the differences in means was applied to data on cage location and microenvironment data of experiments 2 and 3 (significance was defined as $P < 0.01$). A Student's t-test was used to compare ventilation rates of occupied and unoccupied cages. Data from experiment 4 required that the cage contain a litter of pups at the time of monitoring. Consequently the number of samples ranged from 3 to 24 per condition. It was determined that a 21-day interval for bedding changes was unacceptable, and this condition was

Table 1. Effect of cage location in a ventilated rack on ventilation rates*

Cage Location	Left column	Center column	Right column
Row 1, top	63.4 6 1.2	65.7 6 0.5	63.4 6 2.9
Row 5, middle	63.7 6 1.0	63.7 6 1.6	63.9 6 1.4
Row 9, bottom	62.5 6 0.9	65.9 6 0.5	64.7 6 1.0

*Values reported are mean 6 SEM number of air changes/h. n = 3 for each location.

Table 2. Effect of housing of mice on cage ventilation rates*

Cage Location	No Mice	Mice
Row 1, center	65.7 6 0.5	67.2 6 0.2
Row 1, right	63.4 6 2.9	65.8 6 0.9
Row 4, left	63.7 6 1.0	59.8 6 3.0
Row 4, center	63.7 6 1.6	65.6 6 1.1

*Values reported are mean 6 SEM. n = 3 for each location.

omitted from statistical analysis. Because the number of samples in the remaining 6 conditions were highly disparate, Student's t-test ($P < 0.01$) was used for comparison of key variables, rather than evaluation of each condition tested. Regression analysis was used to correlate ammonia concentration with biomass.

Results

Experiment 1; effect of cage location and mice on ventilation: All cages were equally ventilated. Significant differences were not detected among the 9 cage locations tested (Table 1). Mean ventilation rate was 64.1 6 2.3 air changes/h with a 3.6% coefficient of variation. Ventilation rate in 3 of 4 cages was slightly higher when cages were occupied by mice than when unoccupied. These differences, however, were not statistically significant (Table 2).

Experiment 2; effect of increased cage ventilation: Concentrations of ammonia and carbon dioxide in the microenvironment decreased significantly ($P < 0.01$) with increasing ventilation rates (Figure 2a). However, ammonia concentrations were low (< 3 ppm) for all ventilation conditions tested. Relative humidity was significantly higher ($P < 0.01$) for 30 and 40 air changes/h than for other conditions, and relative humidity was not significantly different for 60, 80, and 100 air changes/h (Figure 2b). For ventilation rates of 60 air changes/h or less, microenvironment temperature was 25.0 6 0.02°C. Microenvironment temperature decreased significantly ($P < 0.01$) to 23.3°C when ventilation was increased to 80 air changes/h or more.

Room-ventilation rate remained constant throughout the study, and environment variables did not differ significantly among conditions (data not shown). Significant differences were not detected among conditions in the control cage (data not shown). The following values represent overall means for the experiment. Mean ammonia concentrations ranged from 0.4 to 0.8 ppm in the control cage and room, whereas mean carbon dioxide concentrations were 420 6 3 and 525 6 15 ppm in the control cage and room, respectively. Mean values for the control cage and room, respectively, were determined for relative humidity (46 6 4 and 56 6 2%) and temperature (20.4 6 1.2 and 22.0 6 0.3°C).

Experiment 3; effect of reduced frequency of bedding changes: Mean ammonia concentrations in the cages remained low for 21 days and then increased dramatically to 12 ppm between days 21 and 26 (Table 3). Microenvironment relative humidity was highest (45%) after 21 days of soiled bedding and actually decreased by day 26. Concentrations of carbon dioxide and temperature fluctuated but did not increase as number of days with soiled bedding increased. The environments of the control cage and room remained stable throughout the experiment and were within the range of values reported in experiment 1.

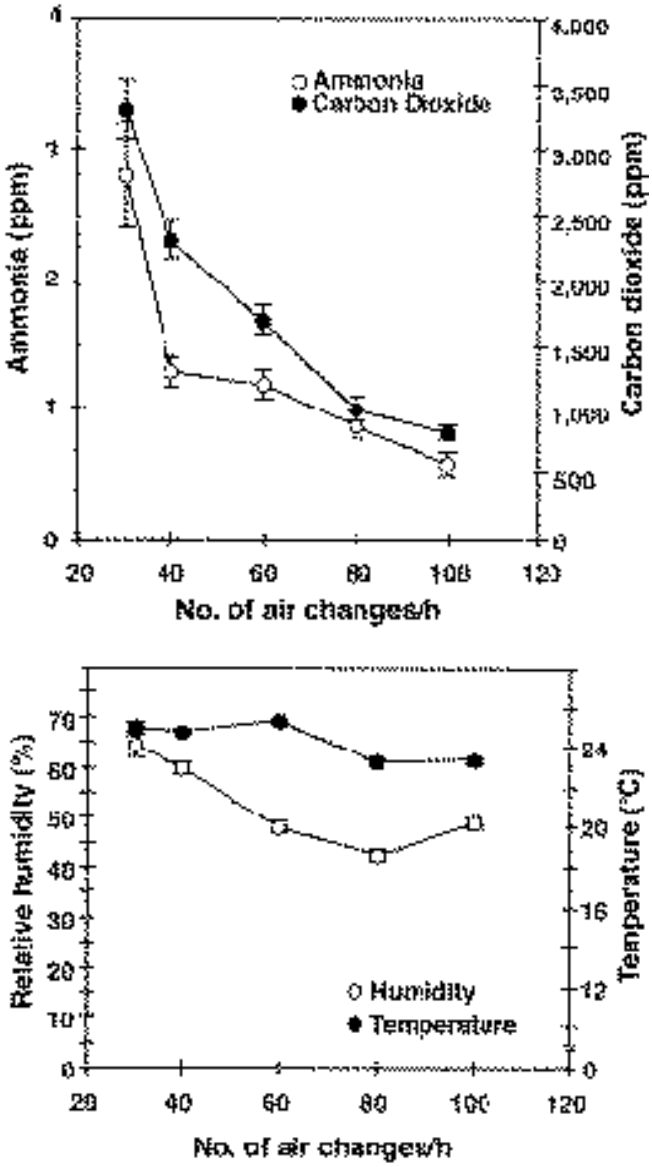

FIG. 2. Intracage ammonia and carbon dioxide concentrations (a) and relative humidity and temperature (b) for various ventilation rates. Measurements were obtained on the 6th day of soiled bedding from 3 occupied mouse cages, using ventilation conditions of 30, 40, 60, 80, and 100 air changes/h. Cage population was 4 adult males.

Experiment 4; effect of ventilation and decreased frequency of bedding changes for breeding colonies: Ammonia concentrations in cages with breeding animals (Figure 3) were higher than those in cages with same-sex adults (Table 3). Although ammonia concentrations for cages housing adult populations were < 3 ppm, those for breeding populations were >25 ppm at times. Mean ammonia concentration increased when soil accumulated for 14 or 21 days (Table 4). Ammonia concentrations were significantly ($P < 0.01$) higher in cages soiled for 14 days, compared with values for those soiled 7 days, for ventilation rates of 30 and 60 air changes/h. For 100 air changes/h, however, ammonia concentrations in cages in which bedding was changed every 14 days were not significantly greater than concentrations in cages at 7 days. Ventilation rate had little effect on cages changed every 7 or 14 days; conditions for 30 air changes/h were not significantly different from 100 air changes/h for 7- or 14-day changing schedules. One condition (30 air changes/h, bedding changed every 21 days), was terminated after completing a single 21-day cycle, because ammonia concentrations in each of these cages ranged from 85 to 185 ppm, cages appeared

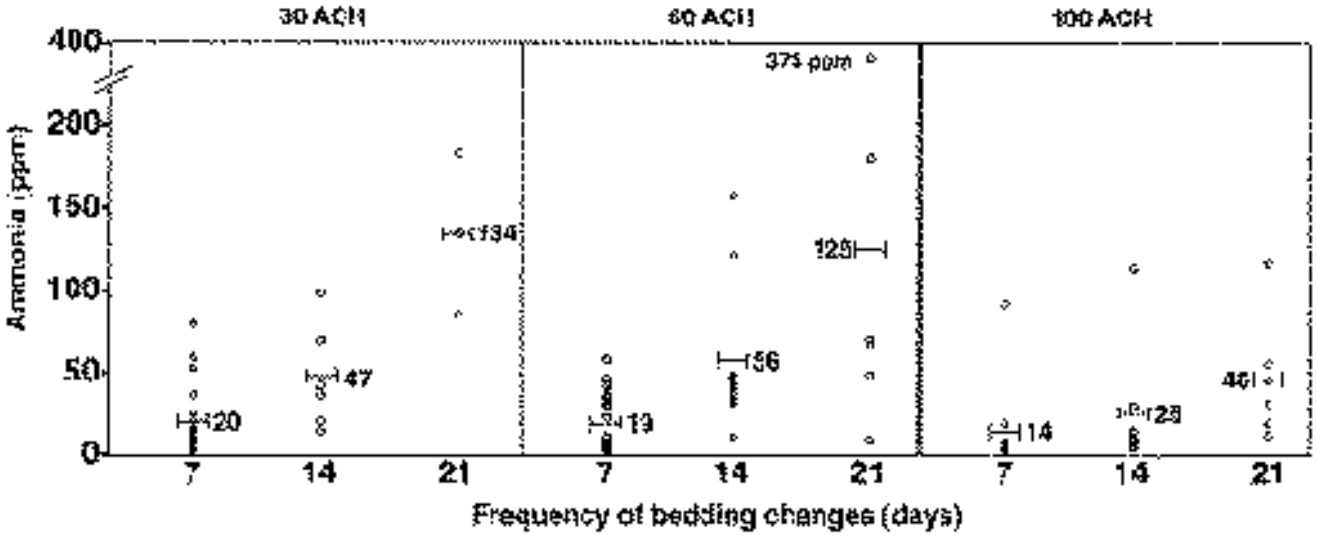

FIG. 3. Ammonia concentrations for various ventilation rates and frequency of bedding changes. During each of the 9 conditions, 3 cages were measured weekly for 6 weeks. Only measurements from the 6th, 13th, or 20th day of soiled bedding are reported. Each cage was occupied with trio-mated breeding adults (2 females, 1 male) and at least 1 litter. Mean number of pups for each experimental condition for the 3 cages ranged from 6.5 to 12 pups/cage. Mean ammonia values are provided for each condition and are represented by a horizontal bar. ACH = air changes/h.

Table 3. Effect of frequency of bedding changes on the microenvironment of 4 adult male mice

	Day of soiled bedding			
	7	14	21	26
Ammonia (ppm)	0.7 6 0.1	1.6 6 0.2	3.0 6 0.4	12.2 6 2.8[a]
Carbon dioxide (ppm)	1,670 6 160	1,765 6 160	1,705 6 180	1,340 6 235[a]
Relative humidity (%)	37 6 1	35 6 1	45 6 1[a]	37 6 1
Temperature (°C)	23.9 6 0.3	24.2 6 0.4	24.7 6 0.3	24.7 6 0.2

n = 12 for each condition. Values reported are mean 6 SEM.
[a]Value is significantly different from other values in same row ($P < 0.01$).
Air change rate of cages was adjusted to 60 6 3 air changes/h.

Table 4. Effect of frequency of bedding changes and number of air changes/h on the microenvironment of breeding trios and their pups

	Day of soiled bedding		
	7	14	21*
Ammonia (ppm)			
30 air changes/h	20 6 5 (18)[†]	47 6 13 (6)	134 6 28 (3)
60 air changes/h	19 6 4 (24)[†]	56 6 12 (11)	125 6 51 (6)
100 air changes/h	14 6 8 (11)	25 6 13 (8)	46 6 15 (6)
Carbon dioxide (ppm)			
30 air changes/h	2,515 6 270[a]	2,345 6 355	3,660 6 420
60 air changes/h	1,960 6 100[a]	2,220 6 170	2,485 6 280
100 air changes/h	1,350 6 80[b]	1,505 6 165	1,785 6 295
Relative humidity (%)			
30 air changes/h	62 6 1[a]	64 6 2[a]	67 6 4
60 air changes/h	51 6 1[b]	53 6 2[a,b]	53 6 5
100 air changes/h	51 6 2[b]	48 6 1[b]	50 6 3
Temperature (°C)			
30 air changes/h	24.6 6 0.4[a,b]	24.0 6 0.2	25.5 6 1.2
60 air changes/h	25.3 6 0.2[a]	25.7 6 0.4	26.1 6 0.8
100 air changes/h	24.2 6 0.2[b]	24.8 6 0.4	24.9 6 0.6

Values reported are mean 6 SEM.
*Condition was deemed unacceptable due to fecal buildup; results were not included in statistical analysis. [†]Within a given variable, values between 7 and 14 day conditions for a specific ventilation rate differ significantly ($P < 0.01$). [a,b]Within a column, values with different superscript letters differ significantly ($P < 0.01$) between ventilation rates for that frequency of bedding change. Values in parentheses represent n, which was the same for all variables within a column.

moist, and mice housed in these cages were not groomed. Although mice housed in the remaining 21-day cycle for bedding changes were groomed, all cages in the 21-day cycle for bedding changes were characterized by substantial fecal accumulation and extremely soiled bedding. Therefore, a 21-day cycle for bedding changes was unacceptable. When bedding changing is reduced to once every 14 days, the highest ventilation rate of 100 air changes/h was necessary to maintain ammonia concentrations at 25 ppm, a concentration that is not significantly different from cages changed once every 7 days. In addition, ventilation rate had little impact on the ammonia concentration in cages changed once every 7 days.

Because the number and age of pups differed among cages, we plotted the data, expressing ammonia as a function of biomass for cages ventilated at 60 air changes/h and changed every 7 or 14 days (Figure 4). Regression analysis indicated that ammonia concentrations were slightly correlated with biomass ($r = 0.57$). Two data points from the 14-day condition were considered outliers, representing significantly higher ammonia concentrations than the remaining measurements. This pattern of outliers can also be seen in our other data (Figure 3). Cages in which ammonia concentrations exceeded 150 ppm had relative humidity of > 61% and a biomass of at least 200 g (3 adult mice and 9 pups). The higher ammonia concentrations could have been the result of a slight leak from a water bottle, which contributed moisture to the cage, in combination with a large number of pups.

Effects of frequency of bedding changes and ventilation rate on carbon dioxide concentration, relative humidity, and temperature were recorded (Table 4). Relative humidity was significantly ($P < 0.05$) higher in cages ventilated at 30 air changes/h than at 100 air changes/h, but remained stable as cages accumulated soiled material for ventilation rates of 60 and 100 air changes/h. Temperature fluctuated by 2°C or less for all conditions.

During experiment 4, cages were ventilated with room air, which resulted in the microenvironment of the empty control cage mirroring that of the room. In the control cages and room, ammonia concentration ranged from 1.2 to 1.3 ppm, carbon dioxide concentration ranged from 480 to 520 ppm, temperature ranged from 21.3 to 22.2°C, and relative humidity ranged from 41 to 45% throughout the experiment.

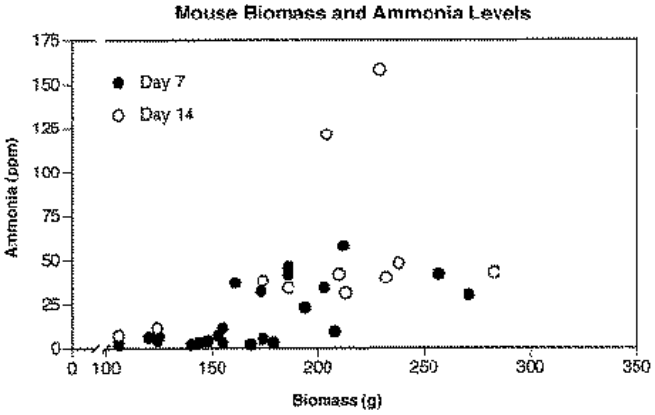

FIG. 4. Ammonia concentrations and biomass in cages ventilated at 60 air changes/h. Ammonia concentration is correlated with biomass in the cage (r = 0.57). Biomass was calculated as the total weight of all mice in the cage at the time of monitoring. Mouse weights were determined on the basis of a scale created by weighing several adult males, adult females, and pups that were from 1 to 28 days old.

Discussion

The recommended range for microenvironment relative humidity is 30 to 70% (23). Relative humidity was within this recommended range during all cage conditions tested in the study reported here. Increased ventilation did maintain a drier cage environment, as documented in experiment 4, in which relative humidity was lower for cages at 60 and 100 air changes/h than at 30 air changes/h. Control of relative humidity by the PIV system was good for ventilation rates of 60 and 100 air changes/h. Relative humidity remained stable as soiled material accumulated from days 7 to 21, helping to reduce the production and accumulation of ammonia. The most important reason for controlling relative humidity in the microenvironment is that increased humidity enhances the proliferation of urease-positive bacteria and results in increased ammonia production (19).

The recommended range of microenvironment temperature for rodents is 18 to 26°C (23). Temperatures were within the upper end of this range at all ventilation rates tested in the study reported here.

Although guidelines for concentrations of carbon dioxide allowable for animals do not exist, and we do not have information on the effects of carbon dioxide on laboratory rodents, the American Conference of Governmental Industrial Hygienists has set the limit of exposure to carbon dioxide for human beings at 5,000 ppm for an 8-h working day (27). Concentrations detected in the study reported here are within the range of previously reported microenvironment concentrations (4,7,15,21) and are within the range generally accepted as harmless for human beings. As expected, carbon dioxide concentrations decreased as ventilation rate increased. It was interesting that carbon dioxide concentrations increased as bedding became soiled; this increase could have resulted from release of carbon dioxide as bacteria broke down fecal material.

The primary source of ammonia in rodent cages is the catalysis of urea in urine and feces by urease-positive bacteria or urease in bedding material (17–20). Guidelines do not exist for exposure of rodents to ammonia, perhaps because the issue of the effects of ammonia on rodents is not clearly elucidated in the literature. Experiments in which rodents are exposed to ammonia typically use 3 sets of alternative conditions. First, rodents are exposed to pure ammonia at a constant concentration or to naturally developing ammonia at a fluctuating concentration. Second, rodents are in contact with bedding and excreta or are suspended above bedding and excreta in mesh cages. Third, rodents are healthy or have been inoculated with infectious agents such as Mycoplasma pulmonis. The effects of ammonia on rodents are strongly influenced by the conditions under which they are exposed. Although it is often stated that ammonia concentrations of 25 ppm are harmful to rodents, that supposition is made on the basis of analysis of results of a study conducted using infected rodents rather than healthy ones (28). In addition, rodents in some studies may have damage from factors other than ammonia, because rodents separated from bedding and excreta by a mesh floor did not have damage when exposed to ammonia concentrations as high as 1,157 ppm (20). We do not have information on the exposure of healthy rodents to naturally developing ammonia concentrations of < 250 ppm. The research community needs studies that use conditions reflecting the typical laboratory environment (i. e., exposure to a natural, gradually increasing source of ammonia; animals that are in contact with soiled bedding; and healthy animals).

Analysis of results of experiment 3 confirmed that growth and accumulation of ammonia concentrating in the microenvironment can be reduced in ventilated cages. In a directly comparable study (21) in which C57BL/6J mice were housed at a rate of 4 males/pen, we detected ammonia concentrations approaching 20 ppm after just 9 days of soiled bedding in an unventilated, bonnet-topped mouse cage. In contrast, with a PIV system set at 60 air changes/h (experiment 2), ammonia concentrations did not approach 10 ppm until cages had accumulated soiled bedding for 26 days. Investigators tested ventilated cages, using the following strains of female mice: Hsd:ICR (24) and Crl:CF-1 BR (3). They documented undetectable or low concentrations of ammonia after 10 and 32 days of soiled bedding, respectively. We conclude that, for certain cage populations housed under appropriate ventilation rates, the frequency of bedding changes can be reduced while maintaining ammonia concentrations of < 25 ppm. Although a decrease in frequency of bedding changes could produce substantial energy and cost savings, further research is required on the biological effects on laboratory animals resulting from a reduced frequency of bedding changes before cost benefits can be fully evaluated.

It is standard husbandry practice to change bedding in PIV cages weekly. Mean ammonia concentrations were < 25 ppm in all cages changed at this frequency in the study reported here (Figure 3). When the frequency of bedding changes for breeding cages was reduced to once every 14 days, mean ammonia concentrations were 25 ppm for 100 air changes/h and approximately 50 ppm for 60 and 30 air changes/h. In cages containing 4 males, ammonia concentrations were much less than 25 ppm when bedding was changed every 21 days. Changing bedding once every 21 days, however, was not acceptable, because the bedding became too soiled.

We concluded that a minimum ventilation rate of 30 air changes/h was adequate for housing same-sex adult populations in cages in which bedding was changed once a week. If brief fluctuations of ammonia concentrations between 50 and 100 ppm are acceptable, then 30 air changes/h was also adequate for breeding cages in which bedding was changed weekly. Ventilation rates of 60 and 100 air changes/h were better for controlling relative humidity than 30 air changes/h. We further concluded that the high ventilation rates of forcefully ventilated PIV systems retard the accumulation of ammonia sufficiently such that the frequency of bedding changes could be reduced to once every 2 weeks. This depends on the assumption that brief fluctuations of ammonia concentrations as high as 150 ppm were acceptable. Because the effect of exposure to naturally developing ammonia and soiled bedding is not well understood, further investigations of the health effects of reduced frequency of bedding changes on animal health are critical.

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