Intra-Cage Air Change Rate on Forced-Air-Ventilated Micro-Isolation System—Environment within Cages: Carbon Dioxide and Oxygen Concentration—

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Abstract: Recently, a forced-air-ventilated micro-isolation system (FVMIS) has been recognized to accurately maintain microenvironmental conditions inside cages, but the details of the relationship between the concentrations of carbon dioxide (CO$_2$) and oxygen (O$_2$) and the air change rate inside the cages have never been reported. In this study, the proper intra-cage air change rate was examined based on the CO$_2$ concentration and O$_2$ concentration inside the cages measured by changing the ventilation volume inside the closed cages of the FVMIS while housing animals. In the experiments, three 8-week-old Wistar strain male rats weighing 303 g on average were housed in each FVMIS cage (capacity: 0.0223 m$^3$), and the temperature, relative humidity, CO$_2$ concentration and O$_2$ concentration were measured when the air change rate inside the cages was varied from 10 air changes per hour (ACH) to 120 ACH. It proved that the CO$_2$ concentration in the FVMIS cages decreased uniformly with the increase in the air change rate. As a result, 60 ACH was required to maintain the CO$_2$ concentration level inside the FVMIS cages equivalent to or less than that in the conventional housing. Otherwise, when based on the O$_2$ concentration, 50 ACH was required. In consideration of these results and others based on ventilation, airflow, temperature and the ammonia concentration reported previously, we concluded that the proper air change rate inside the FVMIS cages should be approximately 60 ACH.

Key words: CO$_2$ concentration, forced-air-ventilation, laboratory animal, microenvironment, O$_2$ concentration

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Introduction

To achieve microbiological control of the laboratory animal housing environment, the micro-isolation system (MIS), which is a shoe box type cage equipped with a hard cover and filter, has been used in U.S.A. The MIS is useful in preventing mutual infection among cages [5], but it has been pointed out that the MIS deteriorates physical environment factors; there is a decrease in intra-cage ventilation [10], and an intra-cage accumulation of moisture, carbon dioxide (CO₂), ammonia and heat [3, 4, 14, 16].

With respect to this point, Keller et al. [9], Wu et al. [16] and Lipman et al. [12] adapted a forced-air ventilation system to this MIS to investigate the control of the intra-cage environment, and proved that such forced-air ventilation was effective in regulating the increase in the ammonia concentration to a great extent. To directly control the climatologic, physical, chemical and biological factors in the microenvironment of animal cages, Kurosawa et al. [11] developed a forced-air-ventilated micro-isolation system (FVMIS) which force ventilated the MIS, and reported that the system could be sufficiently ventilated at 60 air changes per hour (ACH) and that it could suppress the airflow in the cages to under 0.09 m/sec. With the system developed by Kurosawa et al., Yoshida et al. [17] also studied control of the temperature and ammonia concentration under the condition of housing rats (5 per cage, Wistar male rats with a weight of 100 g) so as to evaluate the performance of the FVMIS. As a result, uniform temperature distribution was reported at 65 ACH in the FVMIS examined, and it was found that the FVMIS could suppress the increase in the ammonia concentration compared with the MIS that was a filter capped non-forced-air ventilated system.

Although the FVMIS has forced ventilation which directly affects the gas concentrations inside the cages, few studies on intra-cage CO₂ and oxygen (O₂) concentrations which affect the respiration of the animals housed inside the cages have been reported. Under the condition in which 5 mice were housed in each cage, Lipman et al. [12] measured the CO₂ concentration inside the cage, but the investigation only showed that the FVMIS decreases the CO₂ concentration more than the existing MIS. No suitable ventilation volume was investigated.

The purpose of this study was to investigate, by varying the forced-air ventilation volume, how much ventilation was required in a FVMIS cage with animals in it to maintain its intra-cage CO₂ and O₂ concentration levels equivalent to those in the open cages for common housing on the rack.

Materials and Methods

In this study, the same FVMIS cages as reported by Yoshida et al. [17] were utilized. They were the polycarbonate shoe box-type cages (203 mm H × 483 mm D × 267 mm W, with 922.5 cm² of floor space, DaiDan Co.) with a hard aluminum cover on the top. The hard cover was improved on Yoshida’s et al. by widening the air intake to 50 mm in diameter (19.63 cm² in area). The inner capacity of the cage was 0.0223 m³ with the cover on.

The animals used in this study were Wistar strain (Charles River Japan Inc.) 8-week-old male rats (Rattus norvegicus) with an average weight of 303.3 g. Three rats were housed in each cage. The floor space and the depth of the cage used fulfilled the requirements of the Guide for the Care and Use of Laboratory Animals [7]. Five cages served in this study; 3 of which were FVMIS cages and 2 control cages. Experiments were performed in the Small Animal Laboratory of Animal Center for Medical Research, Okayama University Medical School. The light cycle was 12 hr light and 12 hr dark. Each cage contained 50 g of bedding, that was exchanged every day prior to the experiments. The bedding used was wood shavings (White Flake, Charles River Japan Inc.). The rats were fed a commercial diet (MF, Oriental Yeast Co.) and given tap water ad libitum in water 200 ml bottles.

Temperature, relative humidity, the CO₂ concentration and O₂ concentration inside the room and the cages were measured by varying the ventilation volume inside the FVMIS cages while housing the rats. In this study, only FVMIS cages, not the entire FVMIS including the rack, were used to conduct fine adjustments of the ventilation volume rapidly and precisely. A small Sirrocco fan was used to supply air to the cage by introducing room air and blowing it in through the air intake on the top of the hard cover (Fig. 1). The fan was driven by a 42 W electric motor. Exhaust air from the cage was discharged naturally. In the manner re-
ported previously [11, 17], the intra-cage ventilation was investigated by applying the air change rate which was found by dividing the ventilation volume by cage capacities. The air change rates inside the cages were varied from 10, 30, 45, 50, 60 and 80 to 120 ACH (ventilation volume was 0.22, 0.67, 1.00, 1.12, 1.34, 1.78 and 2.68 m³ per hour, respectively). The ventilation volume was adjusted by measuring the air velocity right under the air intake with a hot wire air velocity meter (MODEL6141, Kanomax Japan Inc.) and by operating the voltage regulator (TCL-150, Toyostar Co.) connected to the electric motor of the air fan. Copper-constantan thermocouples were used to measure the temperature. Intra-cage measurements of temperature were conducted in the center of the cage. A cover tube was attached to each thermocouple to prevent rats from gnawing at it. A hygrometer (HMP233L, VAISALA Co.) was used to measure relative humidity. Intra-cage measurements of relative humidity were also conducted in the center of the cage. A suction gas detector (2LL, 2L, 3lb, Gastex Co.) was used to measure the CO₂ and O₂ concentrations. For CO₂ concentration measurement, 2 types of detectors, one for low concentrations (2LL, measurement range of 300–5000 ppm) and the other for high concentrations (2L, 2500–30000 ppm), were used. The detector for low concentrations was used for 5000 ppm or less. For O₂ concentration measurement, one type of detector (31b, 6–24%) was used. When measuring both CO₂ and O₂ concentrations inside the cages, the detectors were inserted into the cages through a small hole on the side and then air was sucked from the bottom for measurements. The small hole for measurements was closed with adhesive tape when not in use. Temperature and relative humidity inside the cages were measured at intervals of 10 min. CO₂ and O₂ concentrations were measured 60 min after setting the ventilation volume. Room temperature was measured at intervals of 30 min and relative humidity, CO₂ and O₂ concentrations in the room were measured every day prior to the experiments. The environmental parameters in the room were measured at a point approximately 1.5 m high. Measured temperature and humidity were recorded in a data collector (AM-7052 (T), Anritsu Co.). When varying the ventilation volume, a one-minute interval of 200 ACH or equivalent ventilation was provided prior to a new ventilation volume setting. As a control, the same measuring procedure as for the experimental group was done in the open-air cages with the top and sides of the cages shaded by wooden shielding plates to prevent the flow of air from the room diffuser directly entering the cages, in order to imitate conditions in the open-air cages on the rack. When calculating the mean values and the standard deviations of temperature and humidity inside
the cages, data collected during the 30 min after each ventilation volume setting were discarded, so as to prevent the influence of the prior ventilation volume. The parameters used in the calculation were as follows: temperature; n=5–36, relative humidity; n=5–9, CO₂ concentration; n=9–15, O₂ concentration; n=6–9. When comparing each parameter with that of the control, t-test was applied to analyze the mean values statistically (p<0.05).

Results

(1) Temperature
The mean values and the standard deviations of temperature in the room and inside the cages are shown in Table 1. The mean value for the room temperature was 25.1°C. That for the open-air cages (control cages) was 25.4°C which was 0.3°C higher than that for the room. A significant difference was found between them. In the FVMIS cages, the temperature was 27.7°C on average at 10 ACH, the highest value among the present measurements. Furthermore, as the air change rates were increasingly varied to 30, 45, 50, 60, 80 and 120 ACH, the temperature changed to 27.5, 27.3, 26.7, 26.8, 26.6 and 27.0°C, respectively. The correlation coefficient (r) between them was −0.39. They were 1.5–2.6°C higher than that for the room.

(2) Relative humidity
The mean value for relative humidity in the room was the 30.6% shown in Table 1. That for the control cages was 30.8% which was 0.2% higher than for the room. A significant difference was not found between them. In the FVMIS cages, relative humidity was 53.5% on average at 10 ACH, the highest value among the present measurements. As the air change rates were increasingly varied, relative humidity changed to 50.3, 47.5, 43.7, 35.3, 37.5 and 36.2% (r=−0.67). They were 4.7–22.9% higher than that for the room.

(3) CO₂ concentration
CO₂ concentrations both in the room and inside the cages are shown in Fig. 2. The mean value was 604 ppm and the standard deviation was 38 ppm in the room. The mean value was 1744 ppm and the standard deviation was 201 ppm inside the control cages. A significant difference was found between them. In the

| Table 1. Temperature and relative humidity in the FVMIS cages, control (open air) cages and the room |
|-----------------|--------|--------|
|                 | Temperature (°C) | Relative humidity (%) |
| FVMIS cages     |                  |                    |
| 10 ACH<sup>a</sup> | 27.7 ± 0.6<sup>b</sup> | 53.5 ± 8.1 |
| 30 ACH          | 27.5 ± 0.6      | 50.3 ± 7.2       |
| 45 ACH          | 27.3 ± 0.8      | 47.5 ± 6.2       |
| 50 ACH          | 26.7 ± 0.4      | 43.7 ± 5.5       |
| 60 ACH          | 26.8 ± 0.2      | 35.3 ± 3.7       |
| 80 ACH          | 26.6 ± 0.5      | 37.5 ± 4.1       |
| 120 ACH         | 27.0 ± 0.6      | 36.2 ± 3.2       |
| Control cage    |                  |                    |
| open air        | 25.4 ± 0.2      | 30.8 ± 0.7       |
| Room            | 25.1 ± 0.2      | 30.6 ± 3.9       |

<sup>a</sup> ACH: air changes per hour.<sup>b</sup> Each value is the mean ± S.D. (n=5–36 in temperature and 5–9 in relative humidity)

![Fig. 2. CO₂ concentrations in the FVMIS cages, control (open air) cages and the room (n=9–15). *: significantly different from control cages (p<0.05).](image-url)
FVMIS cages, the CO₂ concentration was 7250 ppm on average at 10 ACH, the highest value among the present measurements. As the air change rates were increasingly varied to 30, 45, 50, 60, 80 and 120 ACH, the CO₂ concentrations uniformly decreased respectively to 4638, 3179, 2479, 1877, 1633 and 1442 ppm (r=−0.83). At 80 and 120 ACH, they were lower than in the control cages. When comparing the FVMIS cages with the controls, significant differences were found from 10 to 50 ACH, and also at 120 ACH, but were not found at 60 and 80 ACH.

(4) O₂ concentration

O₂ concentrations both in the room and inside the cages are shown in Fig. 3. The mean value was 21.03% and the standard deviation was 0.23% in the room. The mean value was 20.85% and the standard deviation was 0.10% inside the control cages. No significant difference was found between them. In the FVMIS cages, the O₂ concentration was 20.39% on average at 10 ACH, the lowest value among the present measurements. As the air change rates were increasingly varied, the O₂ concentration changed to 20.61, 20.64, 20.88, 20.98, 20.90 and 21.18% (r=−0.71). From 10 to 45 ACH, they were lower than that in the control cages. When comparing the FVMIS cages with the controls, significant differences were found from 10 to 45 ACH, and also at 120 ACH, but were not found from 50 to 80 ACH.

Discussion

In the FVMIS, determination of the ventilation volume inside the cages is an important factor, since intra-cage environmental control depends on the work of forced-air ventilation. Although insufficient ventilation volume causes deterioration of the intra-cage environment, excessive ventilation not only becomes the cause of wasting energy in the institution, but also deteriorates the housing environment of the laboratory animals [11]. Therefore the establishment of a proper ventilation volume is necessary. To our knowledge, there have been no reports on the proper ventilation volume in the FVMIS. A clear criterion is required to determine the proper air change rates, and the discussion should include the above points. In this study, the necessary air change rates for the FVMIS were examined to build a cage environment almost equivalent to that of open cages. The research was based on the CO₂ concentration, and O₂ concentration which has usually been ignored in previous studies, and on temperature and relative humidity.

The CO₂ concentration in the FVMIS cages decreased uniformly with the increase in the ventilation volume. This shows that forced-air ventilation inside the cages is very useful for diluting and disposing of the CO₂ generated by rats. When a comparison was made of the average CO₂ concentration inside the cages and that of the control, the former values were significantly higher at an air change rate from 10 to 50 ACH, but they were almost the same at 60 ACH, and a statistically significant difference was not recognized, and the former was smaller than the latter at 80 and 120 ACH. Ventilation more frequently than 60 ACH is therefore
considered to be necessary to keep the CO₂ concentration inside the FVMIS cages below the level in a conventional housing environment.

The O₂ concentration in the FVMIS cages tended to become greater with the increase in the air change rate. This shows that forced-air-ventilation is very useful for supplying O₂ to the cages. When a comparison was made of the average intra-cage O₂ concentration and that of the control cages while changing the air change rate, the former at an air change rate of from 10 to 45 ACH was significantly lower than the control value. On the other hand, at an air change rate of from 50 to 80 ACH, no significant difference between the two was recognized, even though the average inside the cages was higher than that in the control, and the former at 120 ACH significantly exceeded the latter. Therefore, in order to maintain an O₂ concentration inside FVMIS cages equal to or more than that in a conventional housing environment, it is considered that an air change rate equal to or more than 50 ACH is required.

Accompanying the increase in the air change rate, there was a tendency to a decrease in the temperature in FVMIS cages, but this tendency was not so clear as in other factors. In the present series of experiments, the whole system of FVMIS was not employed, and ventilation was provided by connecting a small fan to the FVMIS cages. Because of the rather higher temperature in the cages, a possibility cannot be denied that the heat from the Sirocco fan increased the intra-cage temperature. There was a tendency for the relative humidity to decrease as the air change rate increased. This shows that the forced-air ventilation also effectively removes the moisture generated by rats. The humidity is high in MIS cages [3, 16], but, as shown in the present study, the humidity in FVMIS cages is considered to be controllable by providing proper ventilation.

Because no satisfactory study has yet been conducted on the effect of the CO₂ concentration on laboratory animals, a standard value for the CO₂ concentration has not been established not only for a microenvironment but also for a macroenvironment. On the other hand, several standard values have been shown for the CO₂ concentration in a room that is a microenvironment for human subjects. The Building Standards Act in Japan [15] prescribes that the CO₂ concentration must be lower than 1000 ppm in a room. This does not mean the harmful limit for CO₂ itself but means the permissible concentration as the index of pollution when the assumption is made that the physical as well as the chemical properties of air deteriorate proportionally to the increase in CO₂.

In the American Conference of Governmental Industrial Hygienists [2], the maximum permissible concentration of CO₂ in the environment where human beings are exposed for 8 hr per day and 5 days per week is prescribed as 5000 ppm. Further, as to farm animals, Donham [6] researched the environment in the swine housing yard. Based on the dose-response correlation for the swine and workers, he postulates that the recommended maximum concentration of CO₂ in the swine housing yard should be 1540 ppm. Reece et al. [13] reported that a high CO₂ concentration inhibited the growth of broiler chickens. Further, in the field of laboratory animals, it was reported that a CO₂ concentration higher than 5000 ppm had a harmful effect on cats and dogs, using physiologically analytical methods [8]. These results show that the CO₂ concentration is a factor which has grave effects on animals in general, and it is considered that the CO₂ concentration can be used as an index of air pollution in the microenvironment of laboratory animals. It is impossible to apply the standard value for human beings to laboratory animals as it is. It is desirable that the CO₂ concentration inside the cage be at least less than 5000 ppm, when it is considered that the laboratory animal is continually exposed to the microenvironment for 24 hr per day and 7 days per week. Numerous studies have been reported concerning the microenvironment of rodents, but concerning CO₂, detailed studies are few. Even tentatively recommended values have not been reported. It is desirable that researches in this field progress and that a standard for the CO₂ concentration in the microenvironment of laboratory animals be established as early as possible.

In the present study it was shown that it was possible to control the CO₂ and O₂ concentrations in FVMIS cages by forced ventilation, and that more than 60 ACH and 50 ACH, respectively, were necessary in order to keep these concentrations the same as in open cages. In consideration of these results and others based on ventilation, airflow [11], temperature and the ammonia concentration [17] reported previously, the proper air change rate inside FVMIS cages is considered to be approximately 60 ACH. Previously a system in which
over 170 ACH was assumed [9, 16] and a system in
which 23 ACH was assumed to be suitable [12] were
reported, but the former is far greater than the 60 ACH
obtained in this study, and it requires considerable en-
ergy consumption at the institution and also results in
the deterioration of the environment of laboratory ani-
mal institutions [1]. In the design guidelines [11] for laboratory
animal housing, room air velocity is fixed at less than 0.13–
0.18 m/sec, and Kurosawa et al. [11] postulated that
the air velocity becomes 0.09 m/sec in the laboratory
animal dwelling place at 67 ACH. If it is considered
that the air change rate is proportional to the air veloc-
ity, it is anticipated that the air velocity theoretically
becomes 0.23 m/sec in the animal dwelling place at
170 ACH and that the amenity is significantly impaired.
On the other hand, taking into account the present re-
results, a remarkable increase in CO₂ is anticipated at 23
ACH. Twenty-three ACH cannot be considered suffi-
cient, even though a comparison of CO₂ concentrations
cannot be made directly because experimental condi-
tions are different. Compared with that, the 60 ACH
obtained in this study was derived from a comparison
of the results for open cages, and it is considered that it
can be generally applied to the FVMIS, because the
reason for its establishment is clear. In this study, the
experiment was done with cages only, not the whole
system, and over limited time. With an entire system,
long term data should be collected under various breeding
conditions later.

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