Metabolic Cage for Urine Collection from Small Animals with High Level of Performance and Low Cost

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Abstract

The results of designing the original metabolic cage for urine collection from small laboratory animals consisting of a case, a cylindrical animal chamber with the floor, a funnel, a urine collection vessel and two graded drinking bottles that can be placed at a different height depending on animal age are presented. The case was made of laminated particle board; a cylindrical animal chamber was made of polyethylene terephthalate; a circular floor of the animal chamber was made of stainless steel wire cloth mesh. As a funnel for urine collection, a ribbed glass funnel SIMAX (Czech Republic) was used. To prevent rat feces from entering the urine collection vessel, there were installed two stainless steel wire mesh filter discs, namely a larger disc located on the internal ribbed surface of the funnel and a smaller disc located close to the hole of the funnel tube. To support the urine collection vessel, a metal vessel stand with a deepening was made. Between the vessel and the funnel, there was placed a fine stainless steel metal cylinder preventing urine evaporation.

In addition to low cost, the proposed design of the metabolic cage provides high levels of performance as confirmed by its high ability to allow urine to flow freely, as well as to collect urine, significantly smaller volume of urine evaporated, improved housing conditions for animals and allows us to collect the amount of urine more fully reflecting animal diuresis.

Keywords

urine; design; metabolic cage; ability to allow free urine flow; ability to collect urine

Problem statement and analysis of the latest research

Collection of urine from small experimental animals usually requires the use of a metabolic cage [1, 2, 3, 9, 10]. However, the metabolic cages such as Tecniplast (Italy) alongside with other cages manufactured abroad [5, 12, 13, 14, 15, 16] are quite expensive; their cost including the delivery, customs duties and VAT ranges from $1,200 to $2,500. Since at least two metabolic cages are required for the experiment, most small laboratories in Ukraine cannot afford their high cost. Thus, the most affordable metabolic cage is a metabolic cage produced by OpenScience, Russia which costs approximately $700. However, this metabolic cage has a number of significant disadvantages listed below.

One of the main functions of the metabolic cages is their ability to allow flowing urine which is carried out by the bottom of the animal chamber (thereinafter referred to as a floor). The floor of the metabolic cage produced by OpenScience is made of a 4-mm-thick cast acrylic (polymethyl methacrylate or PMMA) sheet (Fig. 1). The gaps with sharp edges and unpolished walls of 5-mm diameter are
designed in such a manner that between the edges of the gaps, there are areas from 4 to 25 mm$^2$ in size, where urine is retained. At a temperature of 20-24$^\circ$C that is required to maintain a laboratory rat, this urine evaporates without passing to the urine collection vessel. The aforementioned disadvantages significantly reduce the ability of the cage to allow urine to flow freely. In addition, such construction of the cage floor prevents large amounts of rat feces from entering a fecal collection vessel.

**Figure 1.** Floor of the animal chamber in the metabolic cage “OpenScience”.

The second main function of the metabolic cages is their ability to collect urine which is carried out by a collection funnel (thereinafter referred to as a funnel). The funnel of the metabolic cage produced by OpenScience is made of polypropylene, the internal surface of which retains urine (Fig. 2). Both rat urine and feces are retained with the mouth of glass separator and its fixation wire that cover the hole of the funnel tube. A drop-shaped structure of glass separator which should separate feces is not the best choice. This function is not carried out. Feces are constantly moistened by urine and stick to the glass surface of the separator. Moreover, its considerable size contributes to rapid urine evaporation.

**Figure 2.** a – Collection funnel (b – its internal surface) and drop-shaped glass separator of the metabolic cage “OpenScience”.

The animal chamber (thereinafter referred to as a chamber) has a number of significant disadvantages as well. In the metabolic cage produced by OpenScience, the surface area of the chamber floor is 298.6 cm$^2$, while the chamber volume is 5823.6 cm$^3$, that does not meet the requirements of laboratory animal maintenance [4, 11, 6, 7, 8]. In addition, the chamber is poorly ventilated as the number and surface area of ventilation holes in its lid are inadequate. The absence of ventilation holes in the lower portion of the chamber prevents air circulation. One drinking bottle fixed too high is not enough if urine is collected from animals with polydipsia.

**The objective of the research** was to develop the design of the metabolic cage for urine collection from small laboratory animals with high level of performance and low cost as well as to test it.
1. **Materials and Methods**

1. Design of the original metabolic cage and its components with the development of a brief technical description and general view drawing, the determination of the materials and the calculations of cage element size, as well as cage size.

2. Testing of the metabolic cage – the determination of daily diuresis and fluid intake in 10 adult male Wistar rats weighing 214-265 g. In order to neutralize individual variation of animals by body weight, the volume of fluid consumed, and the volume of urine excreted, the latter two indicators were calculated per 1 kg of rat body weight \[2\]. Body mass was determined using laboratory scales (table dial scales, GOST 7327-55) \[10\].

3. Statistical processing of the data obtained was performed in R software environment for statistical computing and graphics, version 3.0. \[17\]. Numerical data were expressed as mean ± standard deviation (Mean±SD).

2. **Results and Discussion**

The proposed metabolic cage (a device for urine collection from small animals) was developed by changing the design of the main components of the metabolic cage produced by OpenScience, Russia and replacing the materials they were made of. The proposed metabolic cage consists of the following components (Fig. 3): a cylindrical animal chamber made of polyethylene terephthalate (PET) (1); a metal circular floor of the animal chamber of 267-mm diameter made of stainless steel wire cloth mesh with mesh gap size of 6x6 mm and a wire diameter of 1.20 mm (2); a ribbed glass funnel SIMAX (Czech Republic) (3); two stainless steel wire mesh filter discs to prevent rat feces and hair from entering the urine collection vessel: a larger disc of 185-mm diameter, with mesh gap size of 4x4 mm and a wire diameter of 0.60 mm (4) located on the internal ribbed surface of the funnel and a smaller disc of 47-mm diameter, with mesh gap size of 2.8x2.8 mm and a wire diameter of 0.45 mm (5) located close to the hole of the funnel tube; a case made of laminated particle board (LPB) (6); a fine stainless steel metal cylinder between the vessel and the funnel (7).

**Cylindrical animal chamber** was made of a large bottle used to transport drinking water with a capacity of 19-20 liters. Bottles with elongated middle part were used (Fig. 3; 8). Such bottles have three ridges, namely one ridge along the upper perimeter (Fig. 3; 9) and two ridges along the lower one (Fig. 3; 10). Two lower ridges are separated by the narrowed undersized part as well. These ridges add strength. The bottom (around the lower ridge) and the neck (at the level of bottle shoulder) were cut off. Only bottle body was used; its height may be within the range of 270-370 mm and the lower internal diameter is 270 mm (the value of the upper diameter is of no importance). The volume of such chamber was 14044.4 cm\(^3\) being 2.4 times larger as compared to that in Russian metabolic cage thereby meeting all the requirements of laboratory animal maintenance \[4, 11, 6, 7, 8\]. In the body of the bottle, the following holes were drilled: a) six 12-14-mm holes for placement of the spouts of two graded drinking bottles (Fig. 6); they were placed by pairs at a distance of 120 mm apart and at a height of 60, 90 and 110 mm, respectively (from the lower edge of the case); this allowed regulating the height of placing drinking bottles depending on rat age; b) at least 14-mm holes for air ventilation; they were evenly distributed above the upper edge of metal ring. Chamber lid (Fig. 3; 11) was made of stainless steel wire mesh as in case of its floor. There was no exact shape of the lid; however, it had to be kept closed as adult rats are able to jump to the upper part of the chamber and lift the lid.

The proportion of open space of the animal chamber floor was 69.3% (Fig. 4). According to the results of the colour segmentation method, in case of identical surface areas, open space of the proposed floor was found to be 2.4 times larger as compared to that of the metabolic cage produced by OpenScience, Russia. The floor edge was framed with a 15-mm stainless steel strip at a 3-4-degree angle to the mesh plane in a medial direction using dot welding to fasten it to the wire mesh (slope of edge stripping was required to aid free flow of
Figure 3. Working drawing and general view of the original metabolic cage.

Legend: 1 – cylindrical chamber; 2 – animal chamber floor; 3 – ribbed glass funnel; 4 and 5 – larger and smaller stainless steel wire mesh filter discs in glass funnel; 6 – case; 7 – metal cylinder; 8 – narrowed middle part of the animal chamber; 9 and 10 – upper and lower perimeters of the animal chamber; 11 – chamber lid; 12 – piano door hinge; 13 – backboard of the case; 14 – metal strip for fastening the animal chamber to the case; 15 – stainless steel metal ring; 16 – urine collection vessel; 17 – vessel stand.

urine into the collection funnel). In addition, the proposed floor has a number of advantages over that of the metabolic cage produced by OpenScience: a) it is 3.3 times thinner; however, it does not bend under the weight of up to 900 g; b) the gaps are limited by smooth stainless steel wire of 1.2-mm
diameter that is round in cross-section (in Russian PMMA chamber floor, the gaps have sharp edges and unpolished 4-mm long wall); c) due to large gaps, rat feces freely pass through the mesh floor; d) the chamber floor area is 510.7 cm² exceeding the minimum floor space requirements for 1 rat (350 cm²) [6], being 1.7 times larger as compared to this indicator of the chamber floor of Russian metabolic cage. The aforementioned advantages significantly increased one of the main functions of the proposed device for urine collection from small animals, namely its ability to allow urine to freely pass.

Ribbed glass funnel SIMAX (Czech Republic) made of borosilicate glass (Fig. 5) had a 50-mm long tube, the upper internal diameter of 250 mm (the external diameter – 264 mm) and the lower internal (the diameter of the tube) diameter of 40 mm. Glass smooth and ribbed internal surface of the funnel accelerated the flow of urine.

Two filter discs made of stainless steel wire mesh prevented both rat urine, feces and hair from entering the urine collection vessel. At the same time, a large area of open space of both wire cloths (75.6 and 74.1%) and a small diameter of their wires (0.60 and 0.45 mm) did not affect free flow of urine. The advantages of using a ribbed glass funnel SIMAX and two stainless steel wire mesh filter discs rather than a drop-shaped glass separator significantly increased the second main function of the proposed device for urine collection from small animals, namely its ability to collect urine.

Chamber case was made of LPB 450×320×300 mm in size. The front wall of the body frame, the door, was fastened by means of piano door hinges (Fig. 3; 12); the furniture door magnets were used to hold the door closed. Backboard (600×100 mm) was firmly attached to the back wall of the case (Fig. 3; 13). In the upper wall of the case, the round hole of 257-mm diameter was cut out; the edge bandings were applied; the funnel had to be fitted tightly and freely (without any fastening); the upper edge of the funnel had to be protruded approximately 15 mm from inside the case. Such chamber frame has an aesthetic appeal and it can be easily made in any carpentry workshop.

The animal chamber was fastened to the case by means of 4 thin stainless steel metal strips 18-20 cm in widths, bolts and nuts (Fig. 3; 14), and stainless steel metal ring (Fig. 3; 15) of 252-mm diameter and 35-mm height.

At first, 4 holes were drilled in the metal ring; then, the drilling sites were denoted on the wall of the animal chamber. It was extremely important to place the ring inside the bottle on its undersized part which was between two lower ridges. The lower edge of the metal ring had to be placed 8-10 mm above the lower edge of the animal chamber wall. Then, the holes were drilled in the animal chamber wall. Metal ring has several functions: a) it is a fixture element; b) it directs the flow of urine into the funnel; c) it prevents the tip-over of the chamber floor as the latter is freely placed (without any fastening) on the upper edge of the funnel; such design is of great importance as it allows the floor to vibrate when rats move thereby contributing to a better flow of urine through the mesh floor.

Metal cylinder made of thin stainless steel was of great importance as well; it had to be tightly pulled over the funnel tube. The lower edge of the cylinder was slightly bent outwards to ensure tight attachment to the upper edge of the urine collection vessel (Fig. 3; 16). Such design allowed creating closed space between the vessel and the funnel thereby preventing evaporation of urine when passing to the urine collection vessel from the funnel.
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Figure 5. a - Ribbed glass funnel SIMAX (Czech Republic) (b - its internal surface) fitted in the original metabolic cage.

To support the vessel, a metal vessel stand with a deepening was made (Fig. 3; 17). The fixator for drinking bottles may be easily made of thin stainless steel strip 30 mm in width which should be firmly attached to the back wall of the case as shown in Fig. 6.

To prove high levels of performance of the proposed metabolic cage (a device for urine collection from small animals), an experiment was conducted. During the tests, 42 ml of urine were collected from 6 rats. Two sterilized tubes were equally filled with 21 ml of urine. Using a graduated pipette and a bulb, 3 ml of urine were dropped every hour; there was produced a stream of urine which was directed toward the floor of the animal chamber of the proposed metabolic cage and that of the metabolic cage produced by Russian manufacturer in various directions. On the floor of the animal chamber in the proposed metabolic cage, urine was retained only in the area of mesh nodes (a point or junction where two or more wires meet) as tiny drops that disappeared within one hour. On the floor of the animal chamber in the metabolic cage produced by Russian manufacturer, urine was retained in the areas between the gaps, as well as within the gaps, evaporating within 5-6 hours and more. On the internal surface of the funnel in the proposed device for urine collection from small animals, there was retained a small number of tiny urine drops disappearing in 30-40 minutes. On the internal surface of the funnel in Russian metabolic cage, there were retained large drops and drips of urine being evaporated within two hours. The volume of urine in both vessels was measured in six hours. In the urine collection vessel of the proposed device, 18.1 ml of urine (86.2% of the original volume) were found, while in the

Figure 6. Fixation of drinking bottles and animal chamber to the frame of the original metabolic cage.
urine collection vessel of Russian metabolic cage, there were observed 8.8 ml of urine (41.9%) only, that was 2.1 times less as compared to the proposed metabolic cage.

According to our calculations, the cost of the proposed metabolic cage is approximately $300, that is 2.3 times less than that of the metabolic cage produced by Russian manufacturer.

When testing the original metabolic cage, the volume of fluid consumed by an intact rat (norm) was found to range within 11-19 ml per day constituting (14.9±2.6) ml/day, as well as to be a significantly changeable indicator, as indicated by the coefficient of variation (Cv=17.7–23.1%). The indicator of fluid consumed calculated per 1 kg of rat body weight was determined within 51.4–72.5 ml/kg/day constituting (63.8±8.2) ml/kg/day and was moderately changeable, as confirmed by lower values of the coefficient of variation (Cv=12.7–16.1%).

When studying diuresis, the similar results were obtained. Daily diuresis of intact rats ranged significantly within 4.1–7.6 ml/day, constituting (22.6±4.0) ml/kg/day on average (Cv=15.2–22.9%). Daily diuresis calculated per 1 kg of rat body weight (22.6±4.0) ml/kg/day was less changeable indicator, as confirmed by its coefficient of variation (Cv=9.8–17.8%), ranging within 16.8–30.0 ml/kg/day. When comparing these indicators with those obtained by other researchers [1, 2, 3], in the first case, our data differed by 23-78%, while in the second case, they differed by 4-23% only.

3. Conclusions

1. The proposed metabolic cage (a device for urine collection from small animals) demonstrates high levels of performance as confirmed by its high ability to allow urine to flow freely, as well as to collect urine, significantly smaller volume of urine evaporated, improved housing conditions for animals and allows us to collect the amount of urine more fully reflecting animal diuresis.

2. The cost of the proposed metabolic cage is almost two times cheaper than that of the metabolic cage produced by Russian manufacturer.

3. The proposed metabolic cage may be used for urine collection from small animals (rats, mice, hamsters, guinea pigs, etc.) in biology, veterinary, experimental medicine and pharmacology.

4. Fluid intake and diuresis of intact rats calculated per 1 kg of rat body weight serve as the indicators with significantly lower coefficient of variation and therefore are more reliable relative indicators as compared to their natural analogues. They are relative indicators that are best used when compared with those of experimental animals.

References


