Method for Hand-feeding Mouse Pups with Nursing Bottles

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The purpose of this study was to establish a hand-feeding method for the mouse by using nursing bottles. Five different-sized nipples were tested. The largest nipple used for pups from 12 to 15 days of age was 5.5 mm in diameter and 10 mm long, with a tip of 1.5 mm in diameter. The size of the nipple was much larger than that of the mothers, and bloating due to air accumulation was observed in the artificially-reared pups. To prevent bloating, the nipple size was increased, and a piece of sponge was stuffed into the nipple. Among the 30 pups fed from 1 day of age, 26 were weaned and 4 died. Three pups died due to aspiration of milk into the lung, and the remaining one died due to bloating. The development of nursing bottles with nipples may be potentially useful for the studies of nutrition, behavior, immunology, psychology, and toxicology.

Artificial rearing techniques used for mouse and rat pups include the hand-forced feeding method using a catheter, chronic intragastric cannula method, and automatic feeder. The hand-forced feeding method has been used mainly to establish germ-free colonies of mice and rats (1-4). The chronic intragastric cannula method was originally developed by Messer et al. (5) and was modified later by Hall (6). The procedure of rearing rat pups by feeding them exclusively via chronic intragastric cannulas has been used in many studies of nutrition and behavior (7-12). However, both of these methods have the disadvantage in that they deprive pups of normal behaviors such as suckling and swallowing milk. Furthermore, the chronic intragastric cannula method has the pronounced disadvantage that it requires surgery. For this reason, this method cannot be used during the critical period of 0 to 3 days after birth. The automatic feeder was developed by Hoshiba (13, 14) and allows the rat pups to seek nipples and suckle milk by themselves within 12 h of birth. However we found the automatic feeder difficult to use.

The aim of the present study was to establish a hand-feeding method for the mouse by using a nursing bottle, thereby developing a method that is easily available to many fields of research.

Materials and Methods

Nursing bottle. The nursing bottle was a silicone tube (length, 9 cm; inner diameter, 1.0 cm; outer diameter, 1.4 cm; Fig. 1). A partition was placed in the nursing bottle 2.5 cm from the nipple end. Two silicone tubes (inner diameter, 1 mm; outer diameter, 2 mm; Fuji Systems Corporation, Tokyo, Japan), one for milk inflow and the other for washing the bottle, were inserted into the nipple side of the nursing bottle through the partition. The milk inflow tube was connected to a milk tank. The wash tube (length, 15 cm) was closed with a stopper except when used. In addition, a silicone measuring tube with graduation marks (length, 15 cm; inner diameter, 3 mm; outer diameter, 5 mm) was inserted into the milk inflow tube through the partition to measure the amount of milk consumed. The inner diameter of the nursing bottle was decreased to 6 mm for holding the nipple for the length of 7 mm from the end on the nipple side. The nipple could be removed from the nursing bottle for washing. To prevent milk from flowing backwards and air from flowing into the nursing bottle, a valve was placed at 1 cm from the end of the nipple side. Milk was poured from the milk tank to a reservoir by using a peristaltic pump and then into the measuring tube until the milk reached the marker as well as into the tip of the nipple through the valve. As the pup sucked milk, the level of milk in the measuring tube decreased from the marker. The length from the marker to the point where the level of milk reached was measured, and the weight of milk consumed was calculated from the length. At 7 days of age, the level decreased about 5.0 cm from the marker, so the measuring tube was refilled with milk for each pup.

Nipple. Nipples were made of silicone rubber solutions (KE1950-50A and KE1950-50B, Shin-Etsu Chemical Co., Ltd., Tokyo, Japan). The process of making nipples was the same as that described in the previous study (14), except for the nipple size. Five different-sized nipples (nos. 1 through 5) were used (Fig. 2A); the size was increased with the pup’s age. The smallest nipple (no. 1), used for pups 0 to 3 days of age, was 3 mm in diameter and 6 mm long, and the tip of nipple was 1 mm in diameter. The largest nipple (no. 5), used for pups 12 to 15 days of age, was 5.5 mm in diameter and 10 mm long, with a tip 1.5 mm in diameter. Each nipple had a tube for holding a piece of a 26-gauge needle (length, 10 to 13 mm) for controlling the milk flow rate precisely (Fig. 1). The nipple was stuffed with silicone rubber sponge, which helped to prevent milk from overflowing when the pup attached to the nipple and compressed it.

Milk. Powdered milk for rearing puppies (One Lac Dog Milk, Morinyyu Sunworld Co., Ltd., Tokyo, Japan) was used. The gross composition was as follows: crude protein, > 35%; crude fat, > 34%; crude fiber, < 0.3%; crude ash, < 7.5%; and water, < 7.0%. To approximate the gross composition of mouse milk, the powdered milk (3.6 g) was dissolved in 10 ml hot water. It was freshly prepared just before use at 36°C.
Animals and husbandry. Specific-pathogen-free Jcl:ICR mice (Mus musculus) were purchased from CLEA Japan, Inc. (Osaka, Japan). The source colony was free of the following pathogens: mouse hepatitis virus, Sendai virus, Citrobacter rodentium, Pasteurella pneumotropica, Corynebacterium kutscheri, Pseudomonas aeruginosa, Salmonella spp., Staphylococcus aureus, Mycoplasma pulmonis, Clostridium piliforme, Giardia muris, Spirodictylus muris, Syphacia spp., Aspiculuris tetraptera, and dermatophytes. The mice were bred and used in our conventional facilities. Animals were maintained under the following environmental conditions: lighting, 12:12-h light:dark cycle; temperature, 22 to 25°C; air changes, 12 to 14 per hour; and humidity, 40% to 50%. Sixty pups (1 day of age) were used: 30 pups for feeding experiments, and the remaining 30 pups as controls. Three experiments with 10 pups each were performed. Mice of the experimental group were housed in a cage (230 × 150 × 160 mm) with hard wood chip bedding (Oriental Yeast Co., Ltd., Tokyo, Japan). The cage was kept in a temperature-controlled nursing box, 350 × 350 × 400 mm (temperature, 33 to 34°C; relative humidity, 70 to 80%). Animal care and use were conducted in accordance with the Institutional Guidelines of Okayama University. The experimental protocol was approved by the Institutional Animal Care and Use Committee.

To stimulate both urination and defecation, gentle thumb pressure was applied to the caudal abdomen of pups 30 min before feeding until they were 11 days of age; around 12 days of age, they could urinate and defecate without stimulation. Pups were fed with the nursing bottle 4 times per day (0800, 1300, 1800, and 2000 h). This feeding schedule was determined on the basis of the speed of milk digestion during a preliminary experiment. Pups were allowed to suckle milk ad libitum. The duration of feeding per pup was about 20 sec from 0 to 5 days of age and about 5 min at 16 days of age. Body weights of pups were measured twice a day (0800 and 2000 h) before feeding.

Weaning. In addition to milk, pups were given crushed solid diet (NMF, Oriental Yeast Co., Ltd., Tokyo, Japan) and water in bottles ad libitum from 13 days of age, when they were housed with two 18-day-old mother-reared (MR) pups who had been weaned to the solid diet. The hand-fed (HF) pups were weaned to the solid diet at 15 or 16 days.

Statistics. Body weights and milk consumption are expressed as mean ± standard deviation (SD). An unpaired two-tailed t test was used to make statistical comparisons between the body weights of MR and HF pups. A paired two-tailed t test was used to compare day and night weight gains. All statistical analyses were performed with Excel Statistical Program File (ystat2002.xls; Igaku Tosho Shuppan Co., Ltd., Tokyo, Japan).

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Results
When the nipple was inserted into its mouth, the pup suckled milk almost immediately (Fig. 3). Figure 4B shows the growth curves (mean ± SD) of MR and HF pups on the basis of the body weights measured at 0800 h. The HF pups gained less weight than did the MR pups until 13 days of age. The body weights of 10-day-old MR and HF pups differed significantly (P < 0.01, t test).

The growth rate of the MR pups decreased from 13 days to 17 days of age, then increased again due to the intake of the solid food. The growth rate of the HF pups also decreased after 13 days of age, but the rate of decrease was lower than that of the MR pups. At 16...
days of age, the body weights of the HF pups approximated those of the MR pups, and the weights of 20-day-old MR and HF pups did not differ significantly. Figure 4A shows the body weights measured at 0800 and 2000 h from 10 to 15 days of age. The MR pups gained weight steadily throughout the day and night and had no significant differences in the weight gains between day and night. In contrast, the HF pups gained weight during the day but not during the night because of the lack of feeding. Consequently, as described earlier, total body weight gains per day was greater in the MR pups than in the HF pups.

The daily amount (mean ± SD) of milk consumed per HF pup increased with age until 13 days of age, and then decreased (Fig. 5). The amount of milk consumed per feeding of a representative pup considerably varied throughout the feeding experiment from 24 (1 day of age) to 360 h (15 days) of age. A similar trend was observed in all other pups (data not shown). Around 13 days of age, pups were reluctant to attach to the nipple and began to chew the solid diet and the hard wood chip bedding. Among the 30 pups fed from 1 day of age, 26 were weaned, three died due to aspiration of milk into the lung, and the remaining one died due to bloating.

The HF pups gained less weight than did the MR pups, primarily because of lack of feeding during the night. In addition, the milk substitute used (one for dog pups) may have been difficult for mouse pups to fully digest. The milk given at 2000 h remained undigested in the stomach of pups until next morning, especially in newborn pups. From 9 days of age and older, the pups were able to fully digest the milk that had been given at 2000 h on the previous day. It is necessary to develop a more refined and digestible milk product so that it can be used in many fields of research, including nutrition, immunology, and toxicology.

From 13 days of age, pups were reluctant to attach to the nipple, probably because of fully developed incisors. Around this time, the pups began to chew the solid diet and the hardwood chip bedding. In the case of the MR pups, imitating their mother’s behavior, they began to eat the solid diet. To let them learn from the MR pups, the HF pups were housed with 18-day-old MR pups who had been weaned to the solid diet. The HF pups began to eat the solid diet, imitating the MR pups’ behavior and could be weaned from the nursing bottle to the solid diet at 15 or 16 days of age.

The data obtained in the present experiment is from the ICR mouse strain. During the preliminary feeding experiments using various strains of mice, it appeared that pups born with higher body weight were easier to feed (data not shown). The nursing bottles with nipples described here are easily manipulated by researchers and technicians without special training. This system may be useful for studies of nutrition, behavior, immunology, psychology and toxicology. Moreover, the current method recently has been successfully applied to feeding rat pups by using larger nipples than those used for mice.

**Discussion**

Mouse pups ≤ 10 days of age suckled milk successfully with a nipple shaped similarly to that used for feeding rat pups (Fig. 2B). However, the mice failed to suckle milk once the incisors had developed fully (around 11 days of age), because of air aspiration through the gap between the incisors and nipple. Subsequently, the pups developed bloating due to accumulation of large amounts of air in the stomach (Fig. 7), which could be recognized even through the abdominal wall. Bloating has been observed frequently in artificially reared pups (5, 8, 15, 16). The typical causes of bloating are high osmolality of milk (5, 15), incomplete milk composition (16), and bacterial growth (15). However, the bloat that occurred during the present experiment was due to air accumulation, which was prevented by increasing the size of nipple according to the pups’ ages to fit it to their mouths and by stuffng the nipple with a piece of sponge. As a result, the size of the artificial nipples was much larger than that of mothers’ (Fig. 2A and 3).

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References