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Strain Difference in Coprophagous Behavior in Laboratory Mice (*Mus musculus*)

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ABSTRACT — Coprophagous behavior was observed for a period of 24 hr in two strains of mice (*Mus musculus*), ICR and C3H/He, in a semibarrier-sustained room illuminated for 14 hr a day (lights on at 0500 and off at 1900). The results were compared with those of the IVCS strain (derived from the *dd* strain). Animals of each strain practiced coprophagy frequently for about 8 hr after the lights were turned on. The number of fecal pellets ingested was 6.2 ± 0.6 (mean $\pm$ standard error) in ICR males, 5.3 ± 0.8 in ICR females, 1.7 ± 0.4 in C3H/He males, 1.0 ± 0.4 in IVCS males, and 3.3 ± 0.5 in IVCS females (data on the IVCS strain were quoted from a report by Takahashi *et al.*, Zool. Mag., **92**: 397–401, 1983); there were significant differences among the strains and between the sexes in IVCS mice. Animals displayed two types of coprophagous behavior. In almost all cases, animals ingested feces by taking them directly from their anus, and in rare cases, from the wire-mesh floor. Coprophagous behavior was frequently observed during the time of day when feeding and drinking activity was lower. That is, the behavior of coprophagy was not synchronized with that of feeding and drinking.

INTRODUCTION

A number of nutritional studies on coprophagy have been carried out mainly in rabbits and rats, since Morot [1] reported coprophagy in the rabbit. Many findings on coprophagy have led to the conclusion that coprophagy in rabbits has a significance on the absorbtion of nutrients such as proteins, B vitamins [2], and ashes [3] in the feces. In rats, coprophagy is essential for the utilization of B vitamins, vitamin K, and essential fatty acids synthesized by the bacteria in the large intestine [4]. Coprophagy has also been observed in horses [5], Mongolian gerbils [6], cottontails [7], beavers [8], common shrews [9], and in many other rodents [10], while in mice, which are used for a large variety of biological experiments today, there is only one report on coprophagy by Takahashi *et al.* [11]. We are conducting our course of study on the coprophagy of mice from the viewpoint of behavior and nutrition. In this paper, we will report on the strain differences in coprophagy among the ICR, C3H/He and IVCS [12] strains, and sex differences in IVCS mice.

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MATERIALS AND METHODS

Ten- to 12-week-old mice of the ICR strain (12 males, 9 females) and the C3H/He strain (9 males) were used in the present study. Animals were kept in a semibarrier-sustained room with an ambient temperature of 24 ± 1°C and a relative humidity of 55 ± 5%. The room was ventilated 12 times per hour with all fresh air and illuminated for 14 hr a day (lights on at 0500 and off at 1900). Each animal was housed in a polycarbonate cage

with a raised wire-mesh floor (width 92 mm × depth 205 mm × height 127 mm) and supplied with a pellet diet CA-1 (CLEA Japan, Inc.) and water *ad libitum*. In principle, animals were housed in the cage 5 days before observation. In all animals, body weight, food and water intakes, the number of fecal pellets, and fecal weight were measured for 7 successive days from 3 days before observation. Feces were weighed after drying at room temperature for 24 hr. In each animal, coprophagy, feeding, and drinking behavior were recorded, respectively, for an observation period of 24 hr. Observation in the dark period of the day was made by using an infrared ray-sensitive monitoring apparatus. All observations were carried out as quietly as possible so as not to interrupt the rhythmicities of animal behaviors.

In order to examine sex differences in the frequency of coprophagy in the IVCS strain, 10-week-old male and female animals were gonadectomized under ether anesthesia and their coprophagous behavior was observed for a 24 hr period 3 weeks after the operation. Methods for observation were the same as above-mentioned.

Student's *t*-test was used for statistical analysis of the results except for those represented as rate, to which the chi-square test was applied. Analysis of variance was applied to evaluate the differences between diurnal behavioral patterns.

RESULTS AND DISCUSSION

1. Frequency of coprophagy

Frequency of coprophagy per hour in an individual animal of each strain is shown in Tables 1 and 2, and mean values for 24 hr are given in Table 3.

a) Percentage of animals showing coprophagy All 12 males (100%) and 9 females (100%) in the ICR strain, and 8 males out of 9 (89%) in the C3H/He strain showed coprophagy. In the IVCS strain, 5 males out of 10 (50%), 18 females out of 20 (90%) displayed coprophagy [11]. The rate in the IVCS males was the lowest and statistically significant differences were found compared with that in IVCS females [11] ($P<0.05$), ICR males ($P<0.01$), and ICR females ($P<0.05$), respectively.

b) Frequency of coprophagy in an animal Male animals in the ICR strain ingested 3–10 fecal pellets and the females 2–8. Animals in the C3H/He strain ingested 0–4 pellets, and the IVCS males ingested 0–3, the females 0–8 pellets [11].

c) Mean frequency of coprophagy in each strain (Mean ± standard error) The ICR males showed the highest frequency of coprophagy, 6.2 ± 0.6/24 hr, and this value was significantly higher compared with that of C3H/He males, 1.7 ± 0.4 ($P<0.001$), IVCS males, 1.0 ± 0.4 ($P<0.001$), and IVCS females 3.3 ± 0.5 ($P<0.01$) [11]. The ICR females exhibited a higher value, 5.3 ± 0.8, than that of IVCS males ($P<0.001$), IVCS females ($P<0.05$) [11] and C3H/He males ($P<0.01$). What produced the differences in frequency of coprophagy among the strains or between the sexes in the IVCS strain [11]? Kenagy [10] observed many rodents during coprophagy and found a difference in the frequency of coprophagy; the granivorous kangaroo rat *Dipodomys merriami* rarely ingested feces, but the herbivorous kangaroo rat *D. microps* ingested about 1/4 of the feces excreted in a day. He concluded that coprophagy was more important in herbivorous species. Since the mice used in the present study were of the same species, of the same food habit, and kept on the same diet, none of these factors was considered to be the cause. On the one hand, Coates [13] has reported that the presence of a gut microflora increases the host's basic requirements for some vitamins. That is, considering that the feces contain some vitamins, coprophagy is beneficial not only to the host itself but to the metabolism of bacterial products. It is also known that there are strain differences in the population of bacteria composing a gut microflora [14]. Therefore, it is likely that there are strain differences in the quantity of gut bacteria, vitamins and metabolites produced by the bacteria. The authors, at present, consider the most probable cause of strain difference in the frequency of coprophagy may be the strain difference of the population of gut bacteria and the constitution of gut microflora. On the other

hand, sex differences in the frequency of coprophagy in IVCS mice were examined from the viewpoint of sex hormones and the results are shown in Table 4. The mean frequencies ±standard error were 3.6±0.6 in the intact females, 0.9±0.4 in the intact males, but 5.5±0.8 in females, 3.3±0.8 in males 3 weeks after gonadectomy. In the males, a significant difference was found between the two values ($P<0.05$). Therefore, in the IVCS males, sex hormones were suspected to be involved in coprophagous behavior. Of course, further studies are necessary to demonstrate this point.

d) Rate of coprophagy throughout set periods in a day The rate (no. of mice displaying coprophagy in a pre-set period/no. of mice displaying coprophagy in a day) was compared among three time periods in a day, the early light period (0500–1200), the late light period (1200–1900), and dark period (1900–0500) in each

TABLE 1. Distribution of the number of fecal pellets ingested by male or female ICR mice at different hours of the day

Male Animal No.	Artificial illumination: Light			Dark					Light						Total
1						1	1		2			3		3	10
2										1		1		2	4
3	1		2						1		2		1		7
4										1				2	3
5									2	1				2	5
6										4		4		1	9
7									3		1		1		5
8				1					1				3		5
9			2						2			2		1	7
10									2	2	1	2	1		8
11										1		2		3	6
12			1							2		1		1	5
Time (hr)	1200			1900		2400		0500						1200	6.2±0.6[a]

Vaginal[b] Stage	Female Animal No.	Artificial illumination: Light			Dark					Light						Total
Proestrus	1			1							3				2	6
	2				3								3		2	8
Estrus	3					1				2		1		3		7
Metestrus	4									2						2
	5	3		1						1	1		1	1		8
Diestrus	6												1	1		2
	7		2						1	2	1		1			7
	8										1		1		2	4
	9									3			1			4
Time (hr)		1200			1900		2400		0500						1200	5.3±0.8[a]

a) Value represents mean±SE.
b) Stage at the beginning of coprophagy examination. upper: male, lower: female

TABLE 2. Distribution of the number of fecal pellets ingested by male C3H/He mice at different hours of the day

Animal No.	Artificial illumination			Total
	Light	Dark	Light	
1			1 1	2
2				0
3			1 1	2
4			1	1
5			1 1	2
6	1		2 1	4
7			1	1
8	1		1	2
9			1	1
Time (hr)	1200	1900 2400	0500 1200	1.7±0.4[a]

a) Value represents mean±SE.

TABLE 3. Mean number of fecal pellets ingested per day by ICR, C3H/He or IVCS mice

Strain	Sex	No. of animals	No. of fecal pellets ingested[a]	Statistical evaluations[b]
ICR	male	12	6.2±0.6	f) f) e) e) f) d) d) e)
	female	9	5.3±0.8	
C3H/He	male	9	1.7±0.4	
IVCS[c]	male	10	1.0±0.4	
	female	20	3.3±0.5	

a) Each value represents mean±SE.
b) Significant differences between 2 groups were estimated by Student's *t*-test; d) $P<0.05$, e) $P<0.01$, f) $P<0.001$.
c) Data for the IVCS strain were quoted from Takahashi *et al.* (Zool. Mag., **92**: 397–401, 1983).

TABLE 4. Mean number of fecal pellets ingested per day by normal or gonadectomized female and male IVCS mice

Group	No. of animals	No. of fecal pellets ingested[a]	Statistical evaluations[b]
Female	14	3.6±0.6	d) e) c)
Male	8	0.9±0.4	
Ovariectomized female	6	5.5±0.8	
Castrated male	6	3.3±0.8	

a) Each value represents mean±SE.
b) Significant differences between 2 groups were estimated by Student's *t*-test; c) $P<0.05$, d) $P<0.01$, e) $P<0.001$.

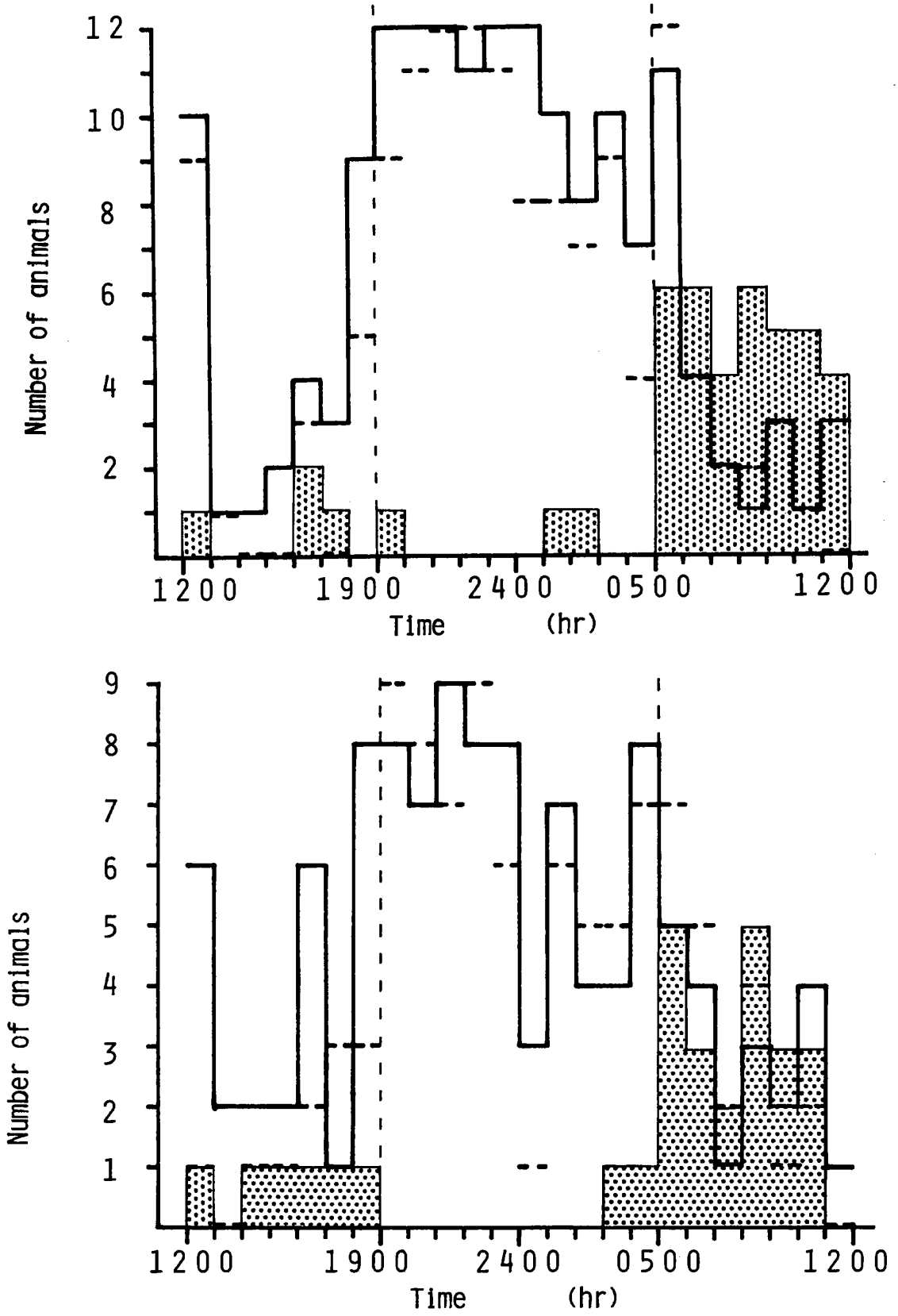

FIG. 1. Appearance of coprophagous, feeding and drinking behaviors during the day in male and female ICR mice. Dotted area, eating of fecal pellet; solid line, eating of food; broken line, drinking of water. upper: male, lower: female.

strain. The ICR males had a significantly higher rate in the early light period than that of the other two periods ($P<0.001$), and the females also exhibited a significantly higher rate in the early light period compared with that of the late light ($P<0.05$) and the dark period ($P<0.001$). The C3H/He males again showed a significantly higher rate in the early light period compared with that of the late light ($P<0.01$) and the dark period ($P<0.001$). The results obtained for the IVCS strain were the same as those in the other two strains; the rate in the early light period was the highest ($P<0.01 \sim P<0.001$) among the three periods [11]. This early light period corresponds to a period of low activity in mice in all the strains. Thus, the tendency for coprophagy to be frequently observed in this period of the day is similar to results obtained from rabbits [15], cottontails [7], common shrews [9], and kangaroo rats [10].

2. Behavioral patterns of coprophagy

Animals displayed two types of coprophagous behavior; in almost all cases, animals ingested feces by taking them directly from their anus, and only rarely feces from the wire-mesh floor. The animals seemed to be aware of feces approaching their anus, and could take a fecal pellet coming out of the anus directly into their mouths. The animals took the pellet, thereafter, with their fore limbs, then usually began to eat it. In some cases, the animals abandoned the grasped pellet without chewing or after several chewings and swallowings. Furthermore, when the feces were excreted in succession, only some of the pellets were eaten, suggesting that there might be a criterion to distinguish the feces to be appropriate for ingestion. As has been described by Kenagy [10], sense of smell, taste, and touch are considered to be factors, but further studies are required to solve this question.

3. The relationship of coprophagy to feeding and drinking

The rate of animals showing coprophagous,

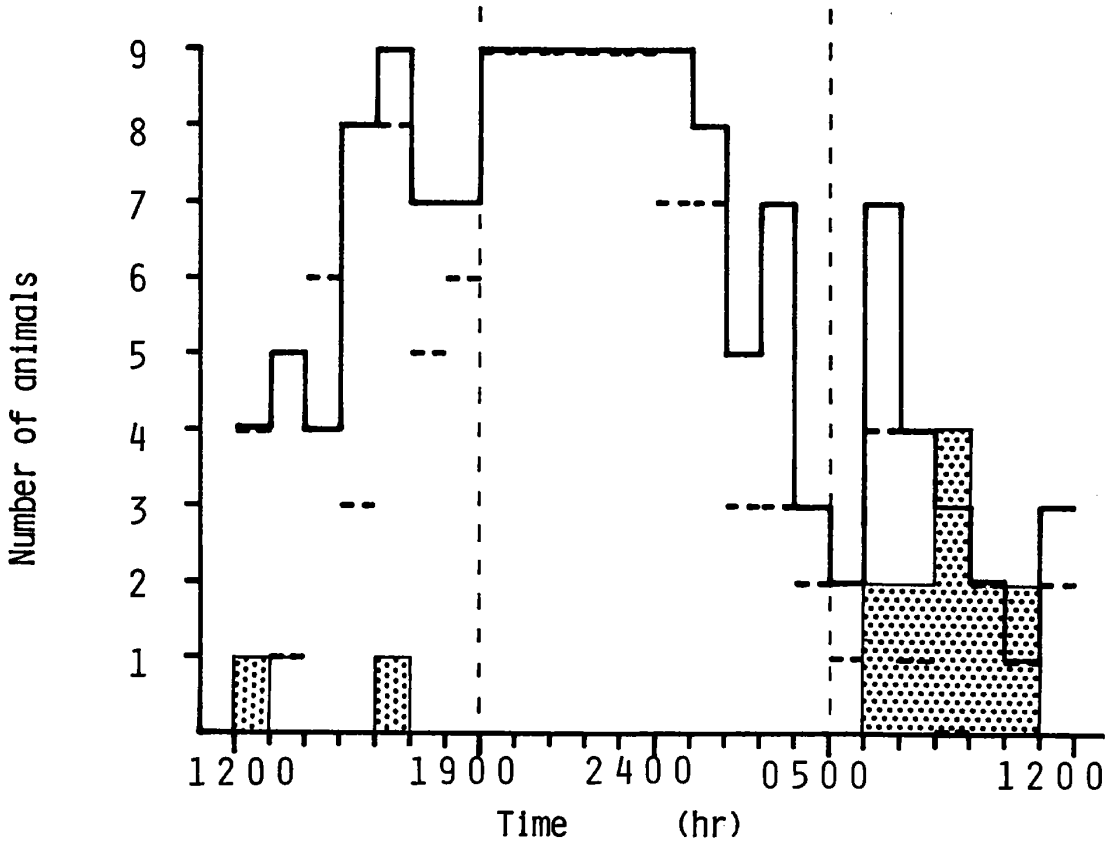

FIG. 2. Appearance of coprophagous, feeding and drinking behaviors during the day in male C3H/He mice. Dotted area, eating of fecal pellet; solid line, eating of food; broken line, drinking of water.

feeding, and drinking behaviors is presented in Figures 1 and 2. The rate of animals showing coprophagy tended to increase in the early light period in each strain. On the other hand, the feeding and drinking activity in animals of both sexes of the ICR strain seemed to be higher between 1900 and 0500. These activities tended to be higher in the C3H/He strain between 1600 and 0200, and around the time the lighting was switched on and off in the IVCS strain [11]. A statistical significance ($P<0.01$) was observed between the diurnal pattern of incidences of feeding and drinking using analysis of variance, but not between coprophagy and feeding or drinking in each strain. This indicates that coprophagous behavior is not synchronized with that of feeding and drinking.

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REFERENCES

1 Morot, C. H. (1882) Des pelotes stomacales des leporides. Mem. Soc. Centr. Med. Vet., **12**: Ser. 1.

2 Huang, T. C., Urich, H. E. and McCay, C. M. (1954) Antibiotics, growth, food utilization and the use of chromic oxide in studies with rabbits. J. Nutr., **54**: 621–630.

3 Thacker, E. J. and Brandt, C. S. (1955) Coprophagy in the rabbit. J. Nutr., **55**: 375–386.

4 Barnes, R. H. (1962) Nutritional implications of coprophagy. Nutr. Rev., **20**: 289–291.

5 Francis-Smith, K. and Wood-Gush, D. G. M. (1977) Coprophagia as seen in throughbred foals. Equine Vet. J., **9**: 155–157.

6 Schwentker, U. (1968) Care and Maintenance of the Mongolian Gerbil, I. L. A. R., National Academy of Science–National Research Council, p. 8.

7 Heisinger, J. F. (1962) Periodicity of reingestion in the cottontail. Am. Midl. Nat., **67**: 441–448.

8 Ingles, L. G. (1961) Reingestion in the mountain beaver. J. Mammal., **42**: 411–412.

9 Loxton, R. G., Raffaelli, D. and Begon, M. (1975) Coprophagy and the diurnal cycle of the common shrew. J. Zool., Lond., **177**: 449–453.

10 Kenagy, G. J. and Hoyt, D. F. (1980) Reingestion of feces in rodents and its daily rhythmicity. Oecologia (Berl.), **44**: 403–409.

11 Takahashi, K. W., Saito, T. R., Suzuki, W., Katsuyama, M., Sakuma, S., Tauchi, K., Kawanishi, H. and Imamichi, T. (1983) Studies on coprophagy in the mouse. Zool. Mag., **92**: 397–401.

12 Nobunaga, T., Takahashi, K. W. and Okamoto, M. T. (1973) Establishment of the IVCS strain mouse; showing a regular four-day estrous cycle. Exp. Anim. (Suppl.), **22**: 277–288.

13 Coates, M. E. (1981) Life without microbes. Lab. Anim., **16**: 189–191.

14 Schaedler, R. W. and Dubos, R. J. (1965) The fecal flora of various strains of mice. Its bearing on their susceptibility to endotoxin. J. Exp. Med., **122**: 59–66.

15 Jilge, B. (1974) Soft feces excretion and passage time in the laboratory rabbit. Lab. Anim., **8**: 337–346.