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Use of Rabbits for GI Drug Absorption Studies: Physiological Study of Stomach-Emptying Controlled Rabbits

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The effect of stomach-emptying control on the physiological state of rabbits was examined. An improved "cangue method" for stomach-emptying control is described and compared with the previously reported "muzzle method."

The increment of rabbit body weight indicated that pre-feeding of the special solid diet to rabbits in both methods did not affect their physiological state. Physiological normality of these rabbits was also confirmed in terms of urine pH and packed cell volume.

The cangue method was found to be superior to the muzzle method as regards easy operation, the prevention of coprophagy and negligible damage to the stomach during the process of gastric lavage.

Analysis of hemocytological and clinico-biochemical data suggested that the physiological state of rabbits is hardly affected by stomach-emptying control using either method.

Keywords——stomach-emptying controlled rabbit; physiological normality; cangue method; muzzle method; animal model; prevention of coprophagy

In our previous paper,[2] a method for simulating the stomach-emptying rate of humans by using rabbits was presented, and the plasma levels of griseofulvin, indomethacin and nalidixic acid in stomach-emptying controlled (SE-controlled) rabbits were compared with those in conventionally fasted rabbits. The usefulness of the SE-controlled rabbit in bioavailability studies was extended to cover different dosage forms, employing three kinds of griseofulvin tablets with different dissolution properties.[3]

The stomach-emptying control of rabbits is characterized by the following four procedures; (1) pre-feeding of a special solid diet for one week before gastric lavage, (2) gastric lavage, (3) prevention of coprophagy and (4) administration of a special soft diet. However, it has not yet been elucidated whether these procedures produce physiological changes in rabbits.

This paper describes the effects of these four procedures on the physiological state of rabbits and also describes an improved method for the stomach-emptying control of rabbits.

Experimental

Materials——Two kinds of solid diet, commercial (CR-1, Nihon Clea Co., Japan) and special (CR-S, Nihon Clea Co.), identical to those described in our previous report,[2] were employed. The special solid diet CR-S was prepared by removing alfalfa from CR-1. A special soft diet was prepared by adding 60 parts of water to 40 parts of CR-S. This mixture was left to stand overnight in a cool place to swell and was then divided into prescribed amounts and made into balls.

Procedure for Stomach-Emptying Control——The stomach-emptying of rabbits was controlled by two methods, that is the previously reported method[2] (muzzle method) and an improved method (cangue method). The two procedures are almost identical except for the method of preventing coprophagy. The procedures are summarized in Table I. Male albino rabbits weighing 2.4—3.4 kg were fed CR-S in place of CR-1 for

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2) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **66**, 69 (1977).
3) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **68**, 1286 (1979).

Table I. Previous and Improved Procedures for Stomach-Emptying Control in Rabbits

Day	Muzzle method (Previous procedure)	Cangue method (Improved procedure)
−7∼−1	Each rabbit was fed 100 g of special solid diet (CR–S).	
−1	Fasted overnight with free access to water	Fasted overnight in a cangue with free access to water
0	Gastric lavage under Na-pentobarbital anesthesia at 9: 00 a.m.	
	Muzzled only during the night (4: 00 p.m.—9: 00 a.m.)	Placed in a cangue throughout the day
1	Each rabbit was fed 200 g of special soft diet at 9: 00 a.m.	
	Muzzled only during the night (4: 00 p.m.—9: 00 a.m.)	Placed in a cangue throughout the day
2	Administration experiment	

one week of conditioning prior to the absorption study. The temperature of the animal room was maintained at 22—26°. In the cangue method, a plastic cangue was placed around the rabbit's neck to prevent coprophagy. The cangue, which consists of two boomerang-like plastic plates (1 mm in thickness and 100 g in weight), is placed around the neck of the rabbit by connecting both ends of the two plates with a clamp screw. In a rabbit fitted with a cangue shaped like a doughnut, having an inner diameter of 5 cm and outer diameter of 28 cm, coprophagy is not possible because the mouth cannot contact the anus, but the animal can drink water freely. Therefore, it is not necessary to limit muzzling during the night and the cangue can be placed around the neck throughout the day. The other procedures used to prevent coprophagy were the same as those described in our previous report.[2)]

Measurement of Urine pH——A urine specimen was drawn from the urinary bladder of the rabbit through urethral catheter and the pH was determined with a pH meter.

Estimation of Coprophagy——The effects of muzzling and the cangue on the prevention of coprophagy were examined using a method identical to that described in our previous paper.[2)] Twelve SE-controlled rabbits (cangue method) were divided into three groups. Each rabbit was housed separately in a cage with a double floor. The upper wire mesh floor was designed to allow feces to drop to the lower floor where they were collected. Gastric lavage was carried out for all rabbits at 9: 00 a.m. Rabbits of the first group were muzzled only during the night (4: 00 p.m.—9: 00 a.m.), whereas a cangue was employed throughout the day for the second group, and the third group was housed without any restriction. Each rabbit was fed 200 g of special soft diet for 24 hr after gastric lavage. The feces were collected and weighed three times during the 48 hr after gastric lavage.

Gross Morphology of the Stomach——After feeding with CR-S for one week, 18 rabbits were divided into two groups. One group of rabbits was conventionally fasted overnight and the other group, fitted with the cangue, was fasted before gastric lavage. Three rabbits belonging to each group of 9 rabbits were killed with an overdose of Na-pentobarbital immediately, 24 hr or 48 hr after gastric lavage. The stomach was quickly removed, the luminal contents were rinsed with saline, the stomach was then resected along the greater curvature and photographs were taken for record.

Hemocytological Analysis——One ml of arterial blood of SE-controlled rabbits was drawn by cardiac puncture 48 hr after gastric lavage. The blood specimen was immediately mixed with 2 mg of EDTA-3K and hemocytological parameters were analyzed using a Technicon SMA 4A autoanalyzer (Technicon Inst. Corp., New York) within 6 hr. Parameters measured were packed cell volume (PCV), hemoglobin concentration, white blood cell count (WBC) and red blood cell count (RBC).

Clinico-biochemical Analysis——Subsequent to the procedure described in the previous paragraph, a further 4 ml of arterial blood was obtained by cardiac puncture 48 hr after gastric lavage. The blood specimen was left to stand at room temperature for 30 min then ice-cooled for 60 min. The specimen was centrifuged and the resulting serum was employed for clinico-biochemical analysis. The following constituents were analyzed, using a Technicon SMA 12/60 multichannel biochemical analyzer (Technicon Inst. Corp., New York): sodium, potassium, calcium, total protein, albumin, glucose, urea nitrogen, uric acid, total cholesterol, alkaline phosphatase, glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase.

Results and Discussion

(A) Effect of the Special Solid Diet

One of the characteristics of our stomach-emptying control method for rabbits is the use of a special solid diet (CR–S) instead of commercial diet (CR-1). As alfalfa was excluded

from CR–S, the crude fiber content of CR–S was reduced to 3.9% from 17.5% of CR-1.[2] According to the review by Adams[4] there is a paucity of literature useful in the choice of fiber content of rabbit diets, though a pelleted feed satisfactory for all classes of rabbits contains about 13%. It was also reported in the review by Yoshida that a fiber content of at least 15% was necessary for rabbits in the growth period.[5]

The effect of feeding CR–S to rabbits was examined by measuring the body weight increment, urine pH and packed cell volume as indices of physiological condition. Eight light rabbits (*ca.* 2.3 kg) and 8 heavy rabbits (*ca.* 2.8 kg) were each divided into two groups. The first of the two groups was fed CR-1 and the second CR–S for four weeks. As shown in Fig. 1, the body weight of the light rabbits increased rapidly up to about 2.8 kg, while that of the heavy rabbits increased slowly during the experimental period. There was no difference in growth rate between rabbits fed CR-1 and those CR–S. After feeding CR–S or CR-1 for 3 weeks, the urine pH and packed cell volume were measured. The results are shown in Table II. No significant difference was observed between the results with the two feeds.

Table II. Effect of Diet on the Urine pH and Packed Cell Volume

Diet	Body weight (kg)	Urine pH	PCV (%)
CR-1	2.84±0.04 [a]	8.54±0.13 [a]	40.9±0.9 [a]
CR–S	2.87±0.06	8.35±0.03	40.5±0.5

Urine pH and packed cell volume (PCV) were measured after feeding the test diet for 3 weeks. Data represent the means±S.E. of 8 rabbits.
a) No statistically significant difference was observed by the t-test ($p>0.2$).

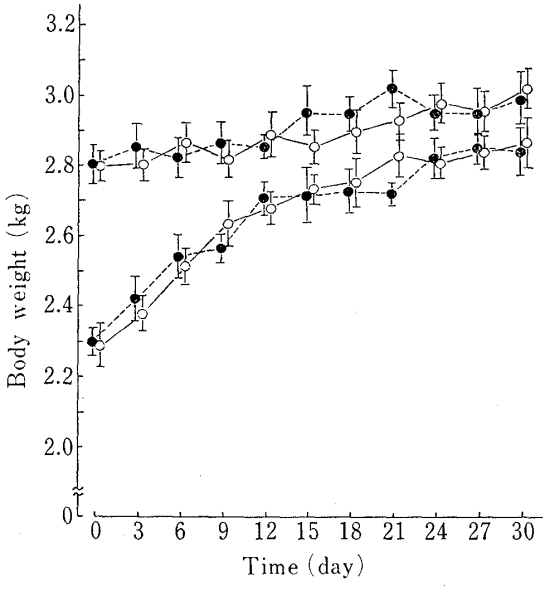

Fig. 1. Growth of Rabbits given Commercial or Special Solid Diet

Light (*ca.* 2.3 kg) and heavy (*ca.* 2.8 kg) rabbits were each divided into two groups and fed 100 g per day of commercial (CR-1, ○) or special solid diet (CR-S, ●) for 4 weeks. Body weight was measured every three days. Each point represents the mean±S.E. of 4 rabbits.

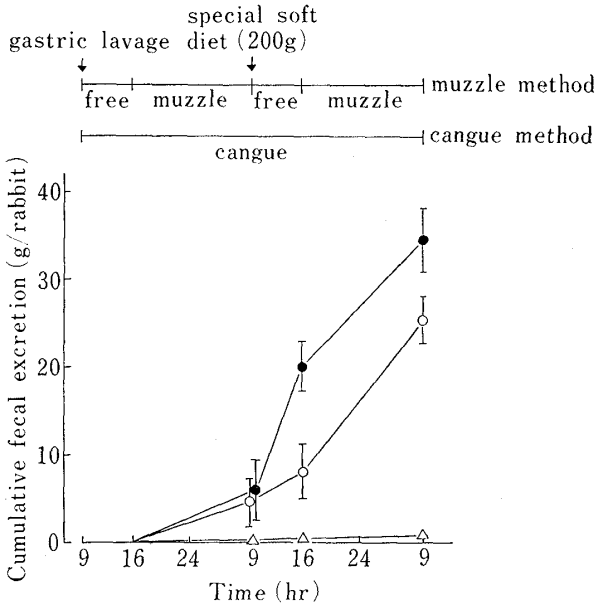

Fig. 2. Prevention of Coprophagy by the Cangue and Muzzle Methods

●, cangue method; ○, muzzle method; △, unrestrained. Each point represents the mean±S.E. of 4 rabbits.

4) C.E. Adams, "The UFAW Handbook on the Care and Management of Laboratory Animals," 4th ed., Longman Group, Edinburgh, 1972, pp. 167—186.
5) T. Yoshida, *Exptl. Animals*, **20**, 47 (1971).

It was concluded that pre-feeding of CR–S in the stomach-emptying control procedure did not significantly affect the physiological condition of the rabbits.

(B) Comparison of the New Stomach-Emptying Control Method (Cangue Method) with the Previous Method (Muzzle Method)

Table I presents an outline of the two methods. The new "cangue method" was characterized by the use of a cangue instead of a muzzle. A plastic collar resembling our rabbit cangue has been used to protect sites of operation[6] and to prevent coprophagy[7] in animal nutrition studies.

In observing the rabbits' behavior, the cangue method is superior to the muzzle method in three respects. First, the rabbit can drink water *ad libitum* and appears to be under little stress and in a more normal condition. Secondly, coprophagy can be completely prevented. Thirdly, the stomach-emptying control procedures are much easier and more clear-cut in the cangue method than in the muzzle method.

Prevention of Coprophagy——Coprophagy was evaluated by the method described previously,[2] and the results are shown in Fig. 2. As expected, the cumulative fecal excretion of rabbits in the cangue method was greater than that of muzzled rabbits, and was in good agreement with that of rabbits muzzled throughout the day.[2] This shows that prevention of coprophagy is more effective by the cangue method, because rabbits can be kept in a cangue throughout the day.

Gross Morphology of the Stomach——One difficulty in the procedure for stomach-emptying control was the possibility that gastric lavage might injure the stomach. Therefore, the stomachs of SE-controlled rabbits using both methods were examined. Examination was performed at three stages of stomach-emptying control, that is immediately after gastric lavage, 24 hr after gastric lavage and 48 hr after gastric lavage according to the stomach-emptying control procedure (Table I). The three stages in both methods are shown in Fig. 3 (typical photos). In photo B a few petechiae, and in photos D and F one focal hemorrhage and a few petechiae were observed at the corps venticuli. This evidence of slight bleeding in the stomach in the case of the muzzle method seems to be attributable to the sucking of the gastric contents through the catheter during gastric lavage.

On the contrary, in the case of the cangue method (photos A, C and E) the stomach did not seem to be injured by gastric lavage, and no difference was observed between the stomach in the case of the cangue method and the untreated normal stomach. This is probably due to the ease of gastric lavage in rabbits in which coprophagy had been completely prevented using a cangue. This suggests that the cangue method is superior to the muzzle method. Gastric bleeding in muzzled rabbits, however, was only slight and did not seem to have any practical affect on drug absorption studies performed 48 hr after gastric lavage.

(C) Comparison of Physiological Parameters of the Blood and Urine between the Stomach-Emptying (SE)-Controlled and Untreated Rabbits

The effects of gastric lavage and the subsequent procedure on the physiological condition of rabbits were also examined in terms of blood and urine parameters. Table III shows the urine pH and PCV before and after gastric lavage in the muzzle method. No change was observed in the PCV of the SE-controlled rabbit up to 48 hr after gastric lavage. These results support the gross morphological observation that the injury caused to rabbits by gastric lavage was negligible even in the muzzle method.

On the other hand, urine pH decreased from 8.6 (just before gastric lavage) to 6.6 (48 hrs after gastric lavage). The physiological value of urine pH in rabbits was reported to be

6) M. Kametaka, *Agr. Bio. Chem.*, **31**, 616 (1967).
7) T. Yoshida, J.R. Pleasants, B.S. Reddy, and B.S. Wostmann, *Br. J. Nutr.*, **22**, 723 (1968).

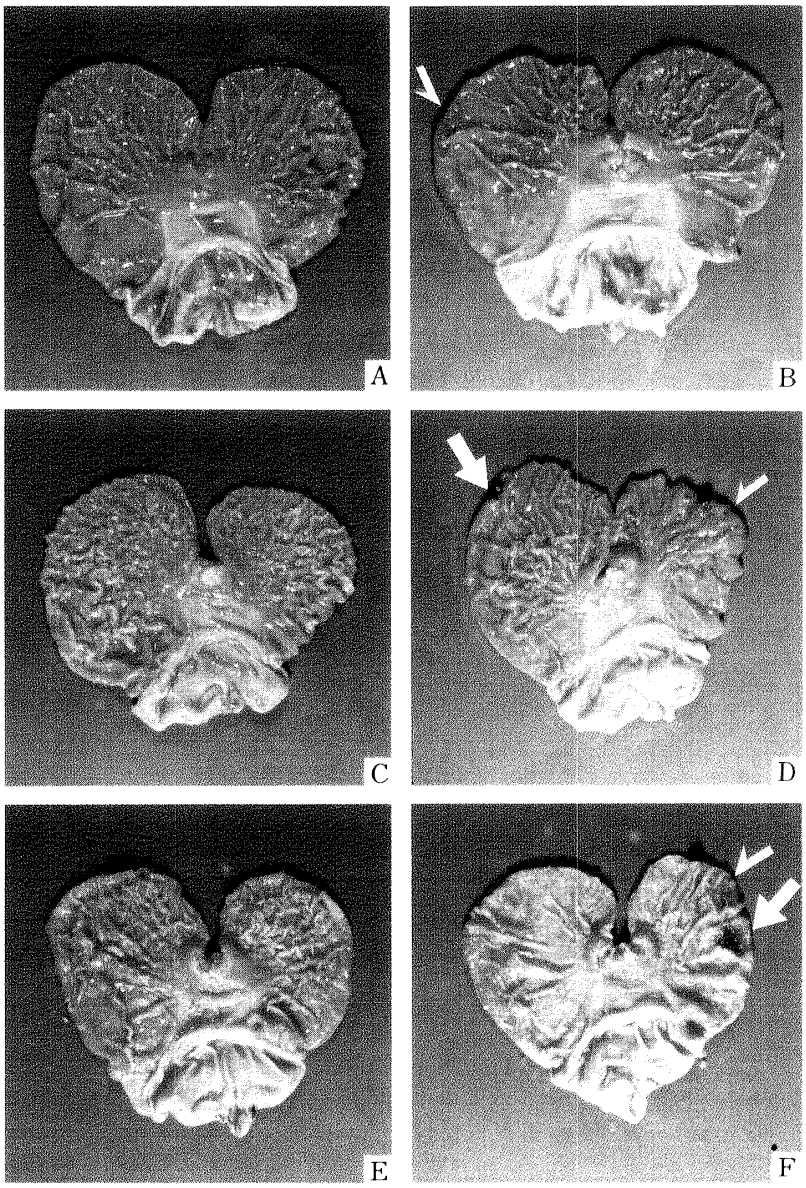

Fig. 3. Gross Morphology of the Rabbit Stomach after Gastric Lavage in the Cangue and Muzzle Methods

Immediately after gastric lavage in the cangue method (A) and the muzzle method (B), 24 hr after gastric lavage in the cangue method (C) and the muzzle method (D), and 48 hrs after gastric lavage in the cangue method (E) and the muzzle method (F).
Arrows: focal hemorrhage (⇦), petechiae (⇦).

TABLE III. Effect of Stomach-Emptying Control on the Urine pH and Packed Cell Volume

Time (hr)	Urine pH	PCV (%)
−1	8.61±0.19	40.4±0.7
2	8.08±0.12	40.5±0.7
24	7.28±0.44	41.9±0.8
48	6.61±0.28	39.6±0.4

Rabbits were controlled by the muzzle method. Gastric lavage was carried out at time 0. Data represent the means±S.E. of 4 rabbits.

Table IV. Hemocytological Parameters in Stomach-Emptying Controlled Rabbits

Rabbit	No.	PCV (%)	WBC $\times 10^{-2}$ (mm^{-3})	Hemoglobin (g/dl)	RBC $\times 10^{-4}$ (mm^{-3})
Untreated	10	39.6±0.9	98.0± 6.2	13.4±0.4	597±38
SE-controlled	9	39.7±1.2	105.0±14.6	13.1±0.4	624±66

Stomach-emptying was controlled by the cangue method. Data represent the means±S.E.

Table V. Values for Various Clinico-biochemical Parameters in the Serum of Stomach-Emptying Controlled Rabbits

Rabbit	No.	Na^+ (meq/l)	K^+ (meq/l)	Ca^{2+} (mg%)	TP[a] (%)	Alb[b] (%)	Glu[c] (%)
Untreated	10	136±1	4.6±0.2	13.4±0.2	6.1±0.1	3.7±0.1	154±2
SE-controlled	9	138±1	4.8±0.1	13.4±0.2	6.0±0.1	3.6±0.1	147±3

Rabbit	No.	UN[d] (mg%)	UA[e] (mg%)	TC[f] (mg/dl)	Al-P[g] (U/l)	GPT[h] (U/l)	GOT[i] (U/l)
Untreated	10	22±2	0.61±0.04	70±6	86±8	44±6	53±9
SE-controlled	9	20±2	0.49±0.05	68±9	63±7	59±11	65±9

a) Total protein.
b) Albumin.
c) Glucose.
d) Urea nitrogen.
e) Uric acid.
f) Total cholesterol.
g) Alkaline phosphatase.
h) Glutamic-pyruvic transaminase.
i) Glutamic-oxaloacetic transaminase. Stomach-emptying was controlled by the cangue method. Data represent the means±S.E.

8.2.[8,9] However, it has also been reported that the urine pH of conventionally fasted rabbits was lower than that of normal rabbits, showing a value of 6 to 7.[10] It is considered that the relatively enhanced protein metabolism caused by starvation increased the urinary acidity of SE-controlled rabbits as well as conventionally fasted rabbits. It is interesting that in some cases the pH of urine of SE-controlled rabbits is approximately equal to the pH of human urine, ranging from 4.8 to 7.6 with an average of about 6.[11] Furthermore, the acidic pH of the SE-controlled rabbit urine must be taken into consideration in the case of a drug whose renal clearance is dependent on the urine pH.

The hemocytological and clinico-biochemical parameters of SE-controlled rabbits prepared by the cangue method were analyzed and are shown in Tables IV and V. All parameters in these tables show that the physiological state of SE-controlled rabbits is the same as that of untreated normal rabbits. The values of all parameters are compatible with those reported previously.[4,8,9]

8) C.S.F. Williams, "Practical Guide to Laboratory Animals," C.V. Mosby, St. Louis, 1976, pp. 148—171.
9) C. Kozma, W. Macklin, L.M. Cummins, and R. Mauer, "The Biology of the Laboratory Rabbit," ed. by S.H. Weisbroth, R.E. Flatt and A.L. Klans, Academic Press, New York, 1974, pp. 50—72.
10) K. Kojima and R. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **22**, 2270 (1974).
11) W.W. Tuttle and B.A. Schottelius, "Textbook of Physiology," C.V. Mosby, St. Louis, 1969, pp. 474—489.

It was concluded that the cangue method is superior to the muzzle method from the standpoint of simplicity and reduced damage to the rabbit stomach. However, the physiological state of rabbits was hardly affected by stomach-emptying control using either method. The results of bioavailability studies employing SE-controlled rabbits obtained by the cangue method will be reported shortly.

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