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Influence of Administration Routes of Antibiotics on the Cecal Flora in Rats.[1] I

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The influence of administration routes of ampicillin (ABPC) on the weight of the cecum and on the cecal flora was investigated in rats. The concentration of ABPC in the cecal contents after single oral administration or multiple oral administration was higher than that after intravenous or rectal administration. However, rather high concentrations of ABPC were observed in cecal contents even after intravenous and rectal administrations. The concentrations of ABPC observed in the cecal contents after administrations by all three routes were larger than the minimum inhibitory concentrations of ABPC for all the cecal flora investigated in the present study. *Enterobacteriaceae* in cecal flora were increased after treatment by all routes of administration and in all dosage schedules. *Staphylococci* were markedly decreased except in the case of single dosage treatment. Populations of other bacteria in the cecal contents were not influenced by administration of ABPC. The route of administration did not appear to modify the influence of ABPC on the cecal flora.

The weight of the cecum after oral administration of ABPC was markedly increased in comparison with that after intravenous or rectal administration.

Keywords——ampicillin; oral ampicillin administration; rectal ampicillin administration; cecal flora; aerobic bacteria; anaerobic bacteria; rectal administration advantage; β-lactamase; cecal enlargement

It is well known that antibiotics such as penicillin G, ampicillin, cephalothin, tetracycline, chloramphenicol and lincomycin cause enteritis and/or diarrhea as side effects, especially in children, when the drug is orally administered.[2-4] It was also reported that enteritis and/or diarrhea following oral administration of antibiotics are mainly due to changes in the intestinal microflora.[5] Further, it is well established that intestinal bacteria play a role in normal physiological processes and contribute to pathological states.[6-8]

Imai *et al.*[9] reported that oral administration of ampicillin (ABPC) and cyclacillin (ACPC) to mice resulted in a decrease of cultured cecal bacteria with an increase of the weight of the cecum. They also reported that a greater influence on bacterial population and on the weight of the cecum was observed following oral administration of ABPC. Since ABPC is less well absorbed from the gastrointestinal tract than ACPC, they considered that the rather large amount of unabsorbed ABPC caused greater changes in bacterial population, resulting in an increase of the weight of cecum. Cecal enlargement has been generally observed after oral administration of antibiotics at high dose in mice.[10-12]

The present report describes the influence of administration route of sodium ampicillin (ABPC Na) on the weight of the cecum and on the cecal flora in rats. Changes of the weight of the cecum and of cecal flora were studied after intravenous, oral and rectal administration of ABPC Na in rats. Cecal microflora were selected because they are thought to be representative of intestinal microflora in rats.[13]

Experimental

Materials——Sodium ampicillin (ABPC Na, 912 μg/mg) was a gift from Kyoto Pharmaceutical Industries Ltd. and was used without further purification. Lauromacrogol (BL-9EX), which was used as an absorption promoter for rectal absorption of ABPC Na, was a gift from Nikko Chemicals. All other reagents were of reagent grade. Culture media used were commercial products as summarized in Table I.

Animal Studies——Wistar male rats weighing 180—200 g were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals. All rats were maintained in laboratory cages at least for one week after purchase. All rats received commercial chow pellets (MF, Oriental Kobo Industries, Ltd.) and water *ad libitum*.

Administration of ABPC Na——Intravenous Administration: ABPC Na was dissolved in saline at the concentration of 50 mg/ml. The solution was administered into the tail vein at a dose of 50 mg/kg.

Oral Administration: ABPC Na was dissolved in deionized water at the concentration of 50 mg/ml. The solution was administered through a stomach tube at a dose of 50 mg/kg.

Rectal Administration: Fifty mg of ABPC Na was well dispersed in a melted mixture of 20 mg of BL-9EX as an absorption promoter and 0.91 g of Witepsol H-15 (Dynamit Nobel Chemicals, West Germany) as a suppository base by sonication in an ultrasonic cleaner for 30 s at 40 °C. The melted suppository was introduced into a glass tube (inner diameter of 9 mm) and kept horizontally for solidification at room temperature. After solidifying, the cylindrical suppository was pushed out of the glass tube and cut into pieces for rectal application. One gram of the suppository/kg body weight (equivalent to 50 mg ABPC Na/kg) was applied to the rectum. After application of the suppository, the anus was closed with a drop of surgical cement (Aronalpha, Sankyo Co., Ltd., Tokyo) to prevent leakage of the melted suppository.

Two-tenths ml of blood was taken form the jugular vein under light anesthesia with ether at appropriate time intervals to determine the concentration of ABPC Na in the blood.

The Weight of the Cecum: The isolated cecum was weighed before and after washing out of the contents.

Cecal Flora Culture——Rats were killed by injection of pentobarbital at 24 or 15 h after the last dosing to calculate the cecal bacterial populations. To avoid exposure of anaerobic bacteria in the cecal contents to air, the cecum was isolated after tightly ligating both ends. The isolated cecum ligated at both ends was sufficiently pressed with fingers to mix its contents well. About 1 g of cecal contents was accurately weighed in a test tube under an N_2 atmosphere and was suspended in 9 volumes of sterilized water containing 1% L-cysteine monohydrochloride.[14] The suspension was diluted by serial 10-fold dilution. Each dilution procedure was performed by mechanical agitation under bubbling of O_2-free CO_2 gas.

One-tenth ml of each dilution was plated onto half of the surface of each selective medium customarily used for the isolation and identification of enteric microorganisms.[14,15] The isolated microorganisms, selective media, and culture conditions are summarized in Table I. Anaerobic microorganisms were incubated by the steel wool method using anaerobic jars (Gidai type, Funatokikai, Gifu).[14] Culture plates for anaerobic organisms were placed in the jars. The jars were evacuated and then filled with N_2 gas. This procedure was repeated four or five times. After the last evacuation, they were filled with 10% CO_2–90% N_2 mixture and incubated for 72 h at 37 °C. The bacterial populations were calculated from the number of colonies developing after incubation.

Analytical Method——Assay of ABPC: The concentrations of ABPC blood and in cecal contents were determined by the usual microbiological disk diffusion method using *Bacillus subtilis* ATCC 6633 as the test

TABLE I. Selective Medium and Incubation Method Used for Examination of Cecal Flora of Rats

Organisms	Medium	Incubation (37 °C)
Enterobacteriaceae	DHL agar (Eiken)	Aerobic, 20 h
Streptococci	Azide blood agar (Difco)	Aerobic, 48 h
Staphylococci	Mannitol salt agar (Nissui)	Aerobic, 48 h
Anaerobic bacteria	Phenylethyl alcohol agar (BBL)	Anaerobic, 72 h[b]
Bacteroidaceae	Bacteroides broth (Nissui)	Anaerobic, 72 h[b]
Lactobacilli	LBS agar (BBL)	Anaerobic, 72 h[b]
Clostridium difficile	Synthetic agar[a]	Anaerobic, 72 h[b]

a) Suzuki *et al.*, Clinical Anaerobic Bacteria Mannual, Nissui Library No. 6, (Nissui Pharmaco., Ltd.).
b) Steel wool method.

organism.[16] In the case of cecal content samples, each sample was sterilized by immersing the test tube containing the cecal content in boiling water for 2 min to prevent interference by the cecal flora. The sterilizing procedure was previously proved (with a spiked sample) not to affect ABPC present in the cecal content.

Susceptibility of Cecal Flora to ABPC: The susceptibility of cecal flora to ABPC was measured by the agar dilution method.[17] Two-tenths ml of cecal content suspension (10%) prepared from cecal contents of untreated rats and saline was plated onto the surface of each selective medium containing ABPC Na in the concentration range of 0.01 to 500 μg/ml. After incubation, the minimum inhibitory concentration of ABPC Na was determined.

Increase of β-lactamase activity after ABPC Na treatment: Instead of direct determination of β-lactamase activity, the stability of ABPC Na in cecal contents obtained from untreated control rats and/or ABPC Na treated rats was determined. ABPC Na was dissolved in the cecal content suspension (1%) prepared with pH 7.4 phosphate buffer at a final concentration of 100 μg/ml. After incubation of the suspension for 2 h at 37 °C, remaining ABPC was determined microbiologically.[16]

Results and Discussion

Concentration of ABPC in Blood and Cecal Contents

ABPC Na was administered to rats by three routes (intravenous, oral and rectal) at the dose of 50 mg/kg/time with three dosage schedules. The time course of the concentration of ABPC in blood after single dosing is shown in Fig. 1. Because of insufficient absorption of ABPC Na from rat rectum, BL-9EX was added in the suppository at the concentration of 2% to enhance the rectal absorption of ABPC Na. The mean peak blood level of ABPC was 1.5 μg/ml for oral administration and 28.5 μg/ml for rectal administration. The extents of bioavailability calculated on the basis of area under the concentration–time course were 6.2 ± 0.8 and 48.5 ± 2.6%, respectively. The large amount of unabsorbed ABPC Na after oral administration was expected to affect the intestinal flora.

The concentration of ABPC in cecal contents was determined at appropriate time intervals after initial administration of ABPC Na. The time courses of the concentration of ABPC in cecal contents after single dosing and multiple dosing are shown in Figs. 2 and 3,

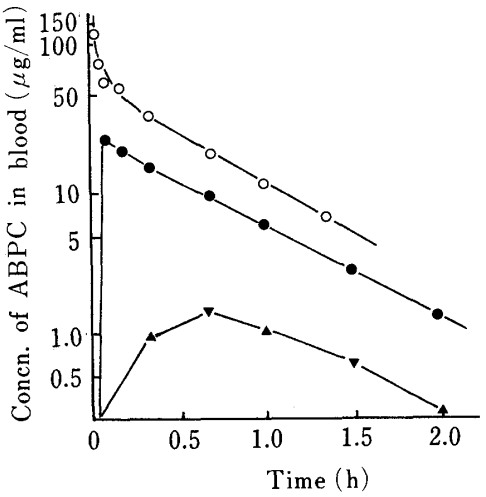

Fig. 1. Blood Levels of ABPC in Rats after Oral (—▲—), Intravenous (—○—), and Rectal (—●—) Administration of ABPC Na at the Dose of 50 mg/kg

Rectal preparation: ABPC Na (5%) and BL-9EX (2%) suspended in Witepsol H-15.
Dose: 50 mg of ABPC Na/kg (equivalent to 1 g of suppository/kg).
Each point represents the mean for four rats. The coefficient of variation of each point is less than 18%.

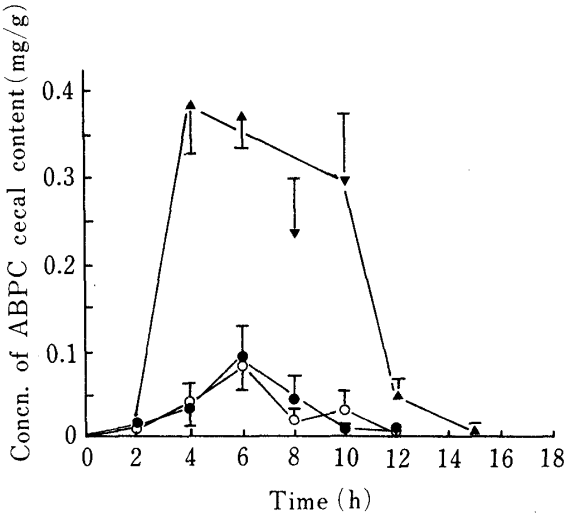

Fig. 2. Concentration of ABPC in Cecal Contents in Rats after Oral (—▲—), Intravenous (—○—), and Rectal (—●—) Administration of ABPC Na at the Dose of 50 mg/kg

Rectal preparation: ABPC Na (5%) and BL-9EX (2%) suspended in Witepsol H-15.
Dose: 50 mg of ABPC Na/kg (equivalent to 1 g of suppository/kg).
Each point represents the mean ± S.E. for 3—4 rats.

respectively. Higher concentration of ABPC was observed in the cecal contents after oral administration than after intravenous or rectal administration. However, a rather high concentration of ABPC was unexpectedly observed in the cecal contents even after intravenous or rectal administration.

A possible reason why a rather high concentration of ABPC Na was observed in the cecum even after rectal administration is spreading of ABPC Na suspended in the suppository to the cecum *via* the colon as reported by Rutten–Kingma *et al.*[18] However, in our experiment, ABPC Na was not detectable by microbioassay in the cecal contents after rectal administration of ABPC Na without absorption promoter (BL-9EX). Thus, ABPC Na in the cecum after rectal administration is not considered to be derived from spreading from the rectum. Other routes such as biliary excretion should be taken into consideration.

Influence of ABPC Na Administered by Three Routes on Cecal Flora

It is considered that the intestinal flora is influenced by age, food intake and seasonal conditions.[19,20] In order to minimize the seasonal influence, the present experiments were all performed within three months from October to December.

Cecal bacterial populations following the administation of ABPC Na (50 mg/kg/time) by three routes with three dosage schedules are shown in Figs. 4—6. The cecal bacterial population was examined 15 or 24 h after final administration of the drug. Among many intestinal bacteria, *Enterobacteriaceae, Streptococci, Staphylococci* as aerobic organisms and *Bacteroidaceae, Lactobacilli* as anaerobic organisms were selected because they predominate in the cecal flora in mice.[9]

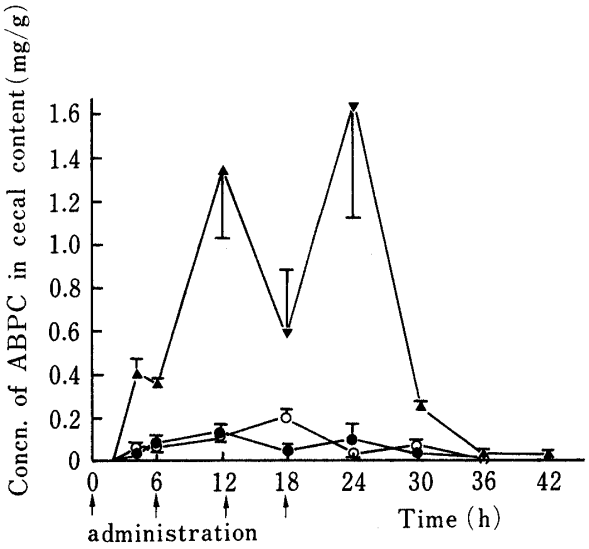

Fig. 3. Concentration of ABPC in Cecal Contents in Rats after Oral (—▲—), Intravenous (—○—), and Rectal (—●—) Multiple Administration of ABPC Na at the Dose of 50 mg/kg/time (4 Times, Every 6 h)

Rectal preparation: ABPC Na (5%) and BL-9EX (2%) suspended in Witepsol H-15
Dose: 50 mg of ABPC Na/kg/time (equivalent to 1 g of suppository/kg/time).
Each point represents the mean ± S.E. for 3—4 rats.

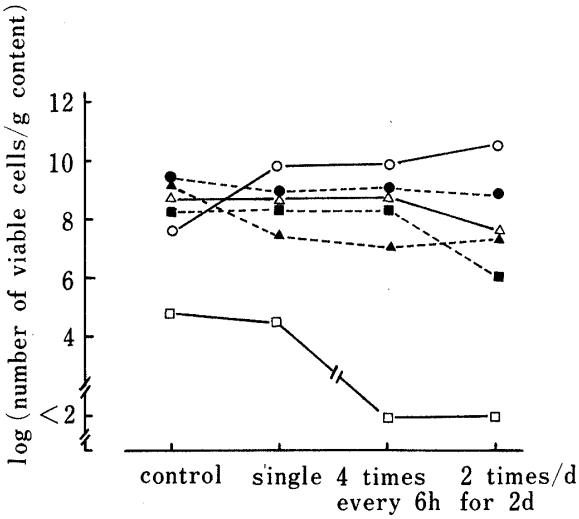

Fig. 4. Changes of Cecal Flora after Oral Administration of ABPC Na at the Dose of 50 mg/kg/time in Rats

(—○—), *Enterobacteriaceae*; (—△—), *Streptococci*; (—□—), *Staphylococci*; (—●—), Anaerobic bacteria; (—▲—), *Bacteroidaceae*; (—■—), *Lactobacilli*.

Cecal flora were cultured at 24 h after final administration in cases of single and multiple (4 times every 6 h) dosing, and 15 h in the case of multiple (2 times/d for 2 d) dosing. ABPC Na was administered at 9:00 AM and 7:00 PM in the case of multiple (2 times/d for 2 d) dosing. Each point represents the mean of the logarithm of the number of viable cells/g content for 6—26 rats. The coefficients of variation of points are less than 9% for *Enterobacteriaceae* and 14% for *Staphylococci*.

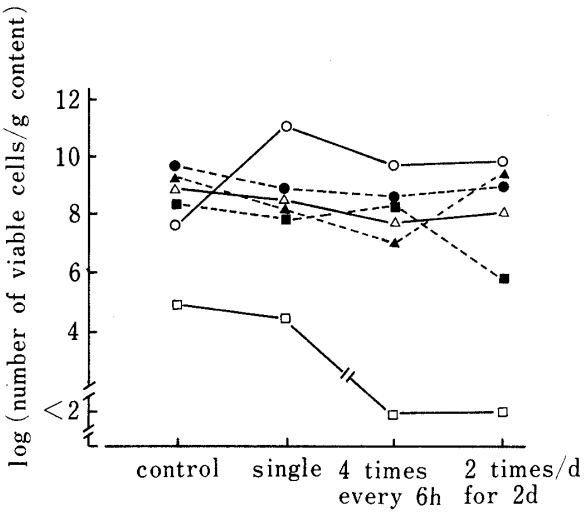

Fig. 5. Changes of Cecal Flora after Intravenous Administration of ABPC Na at the Dose of 50 mg/kg/time in Rats

See Fig. 4 for details. Each point represents the mean of the logarithm of the number of viable cells/g content for 6—26 rats. The coefficients of variation of points are less than 10% for *Enterobacteriaceae* and 2% for *Staphylococci*.

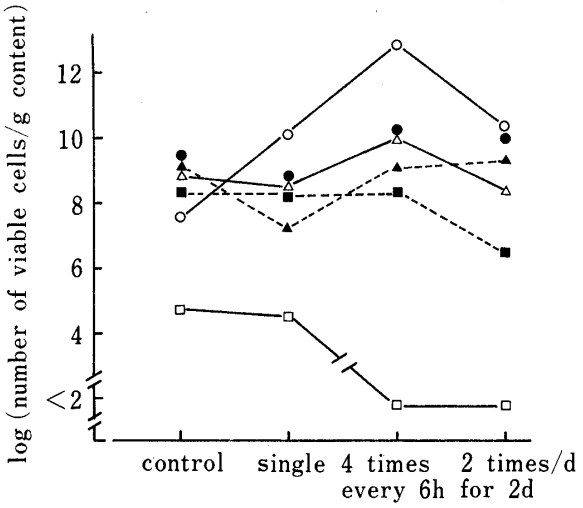

Fig. 6. Changes of Cecal Flora after Rectal Administration of ABPC Na at the Dose of 50 mg/kg/time in Rats

See Fig. 4 for details. Rectal preparation: ABPC Na (5%) and BL-9EX (2%) suspended in Witepsol H-15. Each point represents the mean of the logarithm of the number of viable cells/g content for 6—26 rats. The coefficients of variation of points are less than 11% for *Enterobacteriaceae* and 10% for *Staphylococci*.

Enterobacteriaceae in cecal flora were increased after treatment by all routes of administration and with all dosage schedules. Populations of other bacteria in the cecum (except *Staphylococci*) were not influenced by administration of ABPC Na. The increase in number of *Enterobacteriaceae* after oral administration to mice of antibiotics active against gram-positive strains has been reported by many workers.[4] *Enterobacteriaceae* such as *E. coli* are also well known to produce β-lactamase.[17] Therefore, these results suggest that ABPC-resistant bacteria having β-lactamase activity might have appeared. To confirm this, the stability of ABPC Na in cecal contents separated from ABPC Na-treated animals was determined (Fig. 7). The stability of ABPC Na was decreased in cecal contents separated from ABPC Na-treated rats. The extent of the decrease of stability did not depend on the administration route except in the case of multiple dosing of 10 mg/kg ($p<0.05$). These findings are consistent with the appearance of β-lactamase in the cecal contents after ABPC Na treatment. *Staphylococci* were remarkably decreased except in the case of single dosing treatment.

In two kinds of dosage schedule, a remarkable difference in the concentration of ABPC in the cecal contents was observed between oral administration and intravenous or rectal administration, as shown in Figs. 2 and 3. However, no significant difference of changes of cecal flora after administration of ABPC Na was observed among the different routes of administration as shown in Figs. 4—6.

Even after intravenous or rectal administration, the concentration of ABPC Na in cecal contents was higher than the minimum inhibitory concentration against the bacteria in the cecal flora, as shown in Table II. This may be the reason why no difference of change in cecal flora was observed among the different routes of administration of ABPC Na. Diarrhea observed following oral administration of antibiotics is considered to be due to the increase of *Clostridium difficile* or *Clostridium perfringens*.[4,10,21] In the present study, diarrhea was not observed in rats treated with ABPC Na and *Clostridium difficile* was not detected following

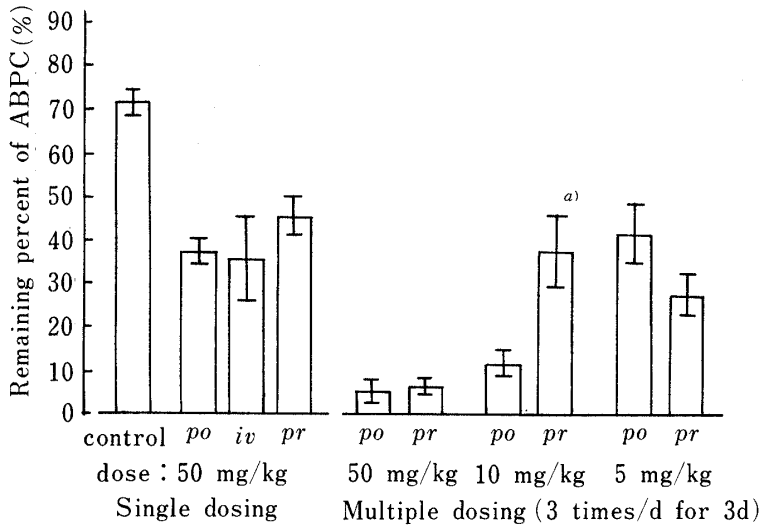

Fig. 7. Stability of ABPC Na in Cecal Content Suspensions Separated from Untreated Control and/or ABPC-Treated Rats

Cecal content was diluted 10-fold with pH 7.4 phosphate buffer (0.1 M), and ABPC Na was dissolved at the final concentration of 100 μg/ml. The cecal contents were obtained at 24 h after single administration, and 15 h after final administration in the case of multiple dosing. ABPC Na was administered at 9:00 AM, 2:00 PM and 7:00 PM in the case of multiple (3 times/d for 3 d) dosing. Each value represents the mean $\pm$ S.E. for 4—5 rats of remaining percent of ABPC Na after incubation for 2 h at 37 °C in cecal content suspension.
a) Significantly different from oral (*po*) in multiple dosing of 10 mg/kg, $p<0.05$.

TABLE II. Antibacterial Spectrum of Ampicillin against Cecal Flora of Rats (Broth Dilution Method)

Enterobacteriaceae	*Streptococci* (β)	*Staphylococci*
6.25	0.78	0.10
Anaerobic bacteria	*Bacteroidaceae*	*Lactobacilli*
50	25	1.56

Each test organism was cultured using selective medium. Inoculation suspension: Two-tenths ml of 10-fold diluted suspension of untreated control cecal contents of rats. Each number represents the MIC (μg/ml) of ABPC.

administration of ABPC Na by all three routes and in all dosage schedules.

Changes of Cecal Weight after Administration of ABPC Na

Changes in the color and weight of the cecum were examined 15 h after the last dosing of ABPC Na. The color of the cecum in ABPC Na treated rats was dark green, very different from the yellow-green in untreated control rats. The cecum was swollen and increased in weight. The results are shown in Fig. 8. The findings are similar to the results obtained by Imai *et al.*[9] and other investigators.[10-12] The weight of the cecum after oral administration of ABPC Na was markedly increased in comparison with that after intravenous or rectal administration. Swelling of the cecum, with increase of weight, is considered to be due to the decrease of fusiform bacteria.[10,12] Fusiform-shaped anaerobes form colonies in layers of mucin covering the epithelium of the cecum and colon.[11]

The present investigation was carried out in the expectation that rectal administration of ABPC Na might show some advantages over oral administration in regard to influence on cecal flora. However, no difference was observed. Unexpectedly, a rather high concentration of ABPC Na in the cecal contents was observed even after intravenous and rectal

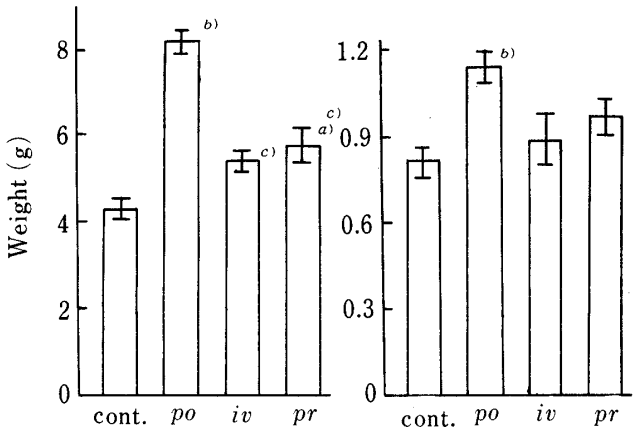

Fig. 8. Changes of Cecal Weight with Contents (Left) and without Contents (Right) after Multiple Dosing of ABPC Na (2 times/d for 2 d) at the Dose of 50 mg/kg/time

ABPC Na was administered at 9:00 AM and 7:00 PM for 2 d. Cecal weight was measured at 15 h after final administration. Each value represents the mean ± S.E. for 6—26 rats.

a), *b*) Significantly different from control, $p<0.05$, $p<0.01$.

c) Significantly different from oral (*po*), $p<0.01$.

administration. ABPC Na in the cecal contents after intravenous and rectal administration is considered to be derived from ABPC Na excreted *via* the bile. The role of biliary excretion of ABPC Na should be further investigated in relation to the influence of drug administration route on the cecal flora.

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