

Studies on Sustained-Release Suppositories. II. Evaluation of Polymer Electrolyte Containing Acidic Groups for Prolonged Rectal Absorption of Bacampicillin in Rabbits¹⁾

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Rectal absorption of bacampicillin hydrochloride (BAPC) was found to show the best bioavailability with Witepsol H-15 as suppository base among various Witepsol bases. However, an effective plasma concentration of drug (above 0.5 $\mu\text{g/ml}$) was only maintained for 2 h, so sustained-release suppositories of BAPC were studied. Bacampicillin reacts with acidic polymer electrolytes such as pectic acid (Pc), chondroitin sulfate (Cd) and precipitates as its adduct with the polymer in an aqueous solution. The dissolution rate of BAPC from the adducts in a solution was slower than that of BAPC itself. The absorptions of BAPC from the suppositories containing the adducts were prolonged, but the bioavailabilities were decreased compared to that from the suppository containing BAPC alone.

Similar prolonged absorption could be obtained simply by mixing Pc or Cd with BAPC in a base. Further, the absorption rate was found to be controlled by the amount of the polymer addition, and both a high plasma level and excellent bioavailability were obtained. This desirable outcome may be due to the simultaneous occurrence of rapid absorption of BAPC itself and formation of the adducts.

Keywords sustained release; bacampicillin; prolonged absorption; adduct; pectic acid; chondroitin sulfate; rectal delivery system; rectal administration; suppository

Suppositories of bacampicillin are preferable to other dosage forms for the avoidance of both the disagreeable bitter taste and first pass effects. In particular, a sustained-release suppository is preferable to conventional suppositories for children and the aged because this characteristic form reduces the necessary frequency of drug administration.

In the previous work,²⁾ we prepared sustained-release suppositories of bacampicillin hydrochloride (BAPC) by using a slightly-water soluble adduct produced between bacampicillin and alginic acid (Alg). Furthermore, we found that similar sustained-release behavior was obtained simply by mixing Alg with BAPC in a suppository base. This effect of Alg addition was assumed to be caused by the formation of the adduct and the slow dissociation of intact BAPC from the adduct, resulting in prolonged absorption. If this is the mechanism of the prolonged absorption, similar results should be available with other polymer electrolytes containing acidic groups in the molecules.

In this study, the interaction of BAPC with pectic acid (Pc) or chondroitin sulfate (Cd) in a solution was investigated and the release behavior and bioavailability of suppositories, which were prepared by using the adducts or by mixing BAPC with Pc (or Cd), were evaluated in rabbits.

Experimental

Materials Bacampicillin hydrochloride (659 $\mu\text{g/mg}$ as ampicillin, Yoshitomi Pharmaceutical Ind., Ltd.) was used as received. Suppository bases such as Witepsol (WP) H-15, S-55 and E-75 were purchased from Maruishi Pharmaceutical Co., Ltd. Hydroxypropyl cellulose (HPC, 150–400 cps), chondroitin sulfate sodium salt (CdNa), and Pc were obtained from Wako Pure Chemical Ind., Ltd. All other chemicals were reagent grade commercial products.

Preparation of Pectic Acid Sodium Salt (PcNa) Sodium hydroxide (1N) was added dropwise to an aqueous Pc suspension (1 g of Pc/100 ml of water) at 0°C with stirring until the suspension became clear. The resultant solution (pH 5.0) was lyophilized.

Preparation of Adducts The adducts were prepared in the same manner as the Alg adduct.²⁾ An aqueous solution of CdNa (2%) or PcNa (2%) was added dropwise to 0.04M BAPC aqueous solution at 0°C under stirring. Each white precipitate was separated by centrifugation and then lyophilized.³⁾ The bacampicillin contents determined by I_2 -colorimetry³⁾ in the adduct with Cd and Pc were $69.1 \pm 1.0\%$ (BAPC–Cd) and $72.5 \pm 1.3\%$

(BAPC–Pc), respectively.

Preparation of Suppository The powder of each adduct, BAPC, PcNa and CdNa, was passed through a no. 150 sieve to obtain particles < 105 μm in size. The conventional suppositories of BAPC were prepared by the fusion method (using various kinds of Witepsol bases and a steel mold). Sustained-release suppositories were prepared as follows: the bases were melted in a beaker on a water bath at 45°C. Then, a mixture of intact BAPC and CdNa (or PcNa) or each adduct was added to the bases and dispersed by sonication with an ultra sonic cleaner (Branson, 220J) for 5 min at 45°C. The melted base mixture was poured into steel suppository molds, which were kept for a while at room temperature and then in a refrigerator at 4°C in order to solidify. The content of BAPC in each suppository was 100 mg per g. Further, as the content was homogeneous throughout a suppository (that is, the BAPC contents of the head and tail of a suppository were found to be 98.7 ± 1.1 and 99.0 ± 0.7 mg/g in BAPC–Pc adduct suppository, and 99.2 ± 0.9 and 98.9 ± 1.2 mg/g in the mixture (BAPC + PcNa) suppository, respectively), no sedimentation of the ingredients occurred during solidification in the mold. These suppositories were stored in a refrigerator until use and were used within 1 week after preparation.

Release of BAPC from Suppositories The release test was carried out according to a modification of the method of Muranishi *et al.*,⁴⁾ using a suitable test instrument (Toyama Industries Co., Ltd., model TMS-103). The test solution was saline (300 ml). A suppository, along with 7 ml of the test solution, was placed in a cylindrical cell equipped with an artificial membrane (Millipore filter, pore size 3.0 μm). The inner test solution of the cell was stirred at 50 rpm, whereas the outer solution was stirred at 100 rpm and 37°C. At appropriate times, 5 ml of the releasing fluid was removed and the same volume of saline was added to the vessel to maintain the original volume. Released BAPC was determined by I_2 -colorimetry.⁵⁾

Measurement of Permeation Profiles The same instrument employed for the measurement of BAPC release from suppositories was used for the measurement of permeation profiles. Powder of BAPC or the adduct, corresponding to 30 mg of the drug, was put into the cell. The other conditions of measurement were the same as for the BAPC release test from suppositories. Bacampicillin in the outer solution was determined in the same manner as above. No change of the solution volume in the cylindrical cell was observed during the measurement.

Rectal Administration White male rabbits weighing 2.5 to 3.5 kg were fasted for 48 h prior to the experiments, but were allowed free access to water. After reducing the suppository size according to the body weight of the rabbits, a suppository containing BAPC at a dose of 17.0 mg/kg was administered into the rectum 3 cm above the anus. A clip was used to prevent expulsion of the suppository for 4 h after dosing. At suitable intervals, a 1 ml blood sample was collected from the ear vein and was centrifuged at 2000 rpm for 20 min.

Measurement of Ampicillin in Plasma The plasma samples were diluted 5–10 fold with water, followed by ultrafiltration using an Air-PRESS-30

(TOSOH Manufacturing Co., Ltd.).⁶⁾ The concentrations of intact ampicillin after administration of BAPC in the resulting filtrates were determined by high-performance liquid chromatography as follows. One hundred microliters of the filtrate was injected into a chromatograph (TOSOH, HLC-803D with a TOSOH, UV-8 model II detector), equipped with a 15 cm \times 4 mm i.d. column, packed with reverse-phase packing substance (TSK gel ODS-80TM, TOSOH Manufacturing Co., Ltd.). The mobile phase was a mixture of 0.01 M phosphate buffer (pH 6.0) and MeOH (70:30) and the flow rate was 0.8 ml/min. The wavelength for the assay of ampicillin was 225 nm at 0.32 AUFS.

Pharmacokinetics Analysis The biological data were analyzed by means of model-independent moment analysis.⁷⁾ The last determined plasma concentration was extrapolated to infinite time based on the terminal slope of the log-time disposition curve. The value of the area under the plasma concentration-time curve ($[AUC]_0^\infty$) and the mean residence time (MRT) of the drug in the body were calculated by means of the trapezoidal rule and by the method of Yamaoka *et al.*,⁸⁾ respectively. The velocity of absorption was evaluated by the Nelson-Wagner method.⁹⁾ Statistical comparison of mean bioavailability and pharmacokinetic parameters was carried out by using the *t*-test.

Results and Discussion

Choice of an Appropriate Suppository Base The rectal absorption of BAPC from Witepsol H-15 base suppository in rabbits was fairly good and the extent of absolute bioavailability was 44%.⁶⁾ The highest bioavailability (88%) was obtained with macrogol 1000 base.¹⁰⁾ This macrogol 1000 base, however, has a strong stimulating effect on the rectum and increases the rectal fluid,¹¹⁾ frequently resulting in diarrhea. Further, the absorption from the suppositories, which contained the BAPC adduct with Alg or the mixture with Alg, formulated with macrogol 1000 base was very poor compared to that from Witepsol H-15 base suppositories.²⁾

Then, suppositories prepared with various kinds of Witepsol bases were examined to select the best suppository base for preparing a sustained-release suppository *in vitro* and *in vivo*. Figure 1 shows the release of BAPC from these suppositories.

Bacampicillin was thoroughly and rapidly released from

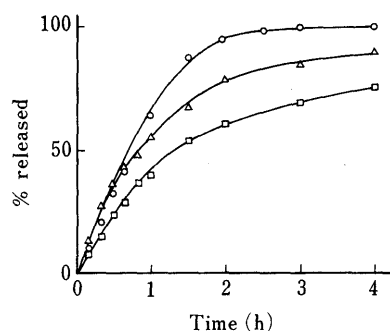


Fig. 1. Release of BAPC from Various Kinds of Witepsol Bases Suppositories

○, H-15; △, S-55; □, E-75. Each point represent the mean of two experiments.

H-15 base suppository as compared with other bases. As shown in Table I, the biological results obtained with these suppositories reflects the relationship of bioavailability parameters to the release behavior *in vitro*; that is, H-15 base simultaneously shows higher plasma peak level (C_{max}) and $[AUC]_0^\infty$ values. Thus, Witepsol H-15 base is most appropriate to prepare the BAPC suppository among the bases tested.

Formation of the Adduct of BAPC with Pectic Acid or Chondroitin Sulfate As described in Experimental, when a solution of PcNa or CdNa was added dropwise into BAPC solution, white precipitates appeared in every case. The precipitate was considered to be a slightly water-soluble BAPC-Pc or BAPC-Cd adduct produced by the binding of BAPC to Pc or Cd, because the solubility of BAPC in aqueous solution decreased with the addition of Pc or Cd (data not shown). The values of BAPC content were determined to be 72.5 ± 1.3 and $69.1 \pm 1.0\%$ in BAPC-Pc and BAPC-Cd, respectively. These values were in good agreement with the values (72.9 and 66.5%) calculated from the molecular weights of BAPC and the constituent saccharide of each polymer.

Figure 2 shows the infrared (IR) spectra of these precipitates, taken with a JASCO DS-701G grating infrared spectrophotometer (and as KBr tablets). Specific absorption bands such as those of ammonium ion due to BAPC at 2600 and 1530 cm^{-1} and carboxylate due to Pc or Cd at 1600 cm^{-1} are observed in each precipitate. From these results, each precipitate was concluded to be an adduct (BAPC-Pc or BAPC-Cd), in which an amino group of BAPC and a carboxyl group of the polymer combine directly to produce a slightly water-soluble salt.

Permeation Profiles The permeation behavior of BAPC and its adducts through a membrane from a small amount of saline solution into a large volume of saline solution is shown in Fig. 3.

As expected from the results on the adduct with Alg,¹⁰⁾ BAPC permeated more slowly from the adduct with Pc (or Cd) than from BAPC itself.

Release Behavior of BAPC from Suppositories Containing the Adduct and the Physical Mixture of Intact BAPC and Sodium Salt of Polymer The release patterns of BAPC from Witepsol H-15 suppositories containing intact BAPC or BAPC-Pc (or BAPC-Cd) adduct in saline solution are shown in Fig. 4a.

The release rates from the suppositories containing the adducts were apparently decreased, and this was assumed to be attributable to the slow permeation profiles shown in Fig. 3. Further, these release rates did not change after storage of the suppositories for one month at 4 °C. If BAPC and the polymer form the adduct immediately upon dissolution of the suppository containing the mixture of both

TABLE I. Bioavailability Parameters of Bacampicillin (Dose 17.0 mg/kg) in Rabbits after Rectal Administration of Various Kinds of Suppository Bases

Suppository base	C_{max}^b ($\mu\text{g/ml}$)	t_{max}^b (min)	MRT^b (h)	$[AUC]_0^\infty^b$ ($\text{h} \cdot \mu\text{g/ml}$)	EBA ^c (%)
PEG ^{a)}	5.07 ± 0.31	36.7 ± 3.8	8.02 ± 2.22	15.90 ± 1.90	87.9
WP H-15 ^{a)}	6.95 ± 0.25	17.1 ± 4.1	1.42 ± 0.35	8.00 ± 0.74	44.2
WP S-55	3.48 ± 1.22	11.7 ± 2.9	0.94 ± 0.20	5.52 ± 0.13	30.5
WP F-75	1.89 ± 0.82	17.5 ± 2.5	0.71 ± 0.12	2.19 ± 0.82	12.1

a) PEG: polyethylene glycol. Taken from ref. 10. b) Each value represents the mean \pm S.D. of four rabbits. C_{max} and t_{max} were extracted from a table of peak concentrations. c) EBA: extent of bioavailability: $[AUC]_0^\infty \text{ rect.} / [AUC]_0^\infty \text{ i.v.}$

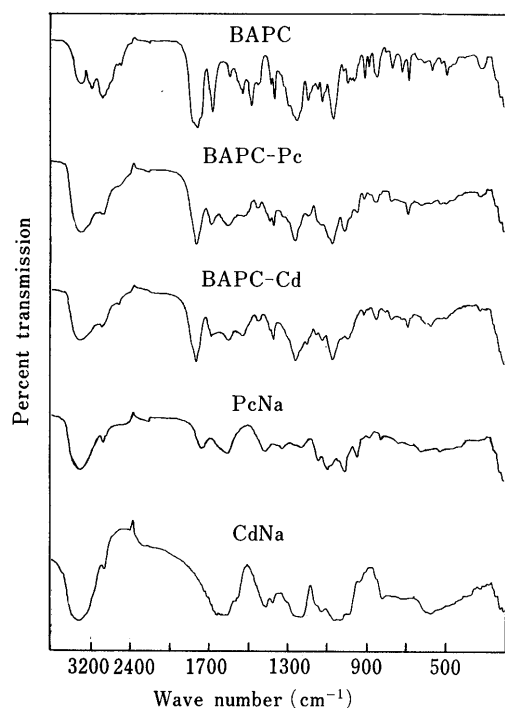


Fig. 2. IR Spectra of Intact BAPC, Precipitates and Polymers

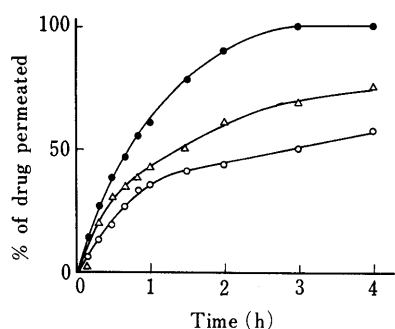


Fig. 3. Permeation Profiles of BAPC from BAPC-Pc or BAPC-Cd Adducts and Intact BAPC

●, BAPC; ○, BAPC-Pc; △, BAPC-Cd. Each point represents the mean of two experiments.

substances in the test solution, the release of BAPC may likewise be delayed. As we would expect, the release profile of BAPC from each suppository containing the mixture agreed very closely with that obtained from the adduct suppository (Fig. 4b). From the above results, the release rate of BAPC from suppositories was found to be controllable by the addition of a polymer in the same way as by using the adduct.

Rectal Absorption of BAPC from Suppositories Containing Adducts The mean plasma concentrations of ampicillin after rectal administration of BAPC-Cd or BAPC-Pc adduct suppositories (Witepsol H-15 base) in rabbits are shown in Fig. 5. The absorption after administration of each adduct suppository was poor compared to that after administration of a suppository containing only BAPC.

The C_{max} value was significantly lower ($1.29 \pm 0.48 \mu\text{g/ml}$ for BAPC-Pc, $2.30 \pm 0.98 \mu\text{g/ml}$ for BAPC-Cd) and the time to peak (t_{max}) was delayed significantly (46.7 ± 13.1 min for BAPC-Pc, 36.7 ± 5.8 min for BAPC-Cd) compared to the values of BAPC alone ($6.95 \pm 0.25 \mu\text{g/ml}$

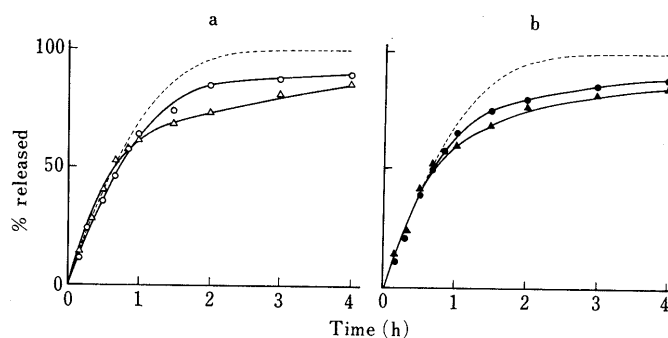


Fig. 4. Release of BAPC from Witepsol H-15 Suppositories Containing Adducts (a) and the Physical Mixtures of Intact BAPC and the Polymer (b)

-----, BAPC alone; ○, BAPC-Pc adduct; △, BAPC-Cd adduct; ●, BAPC + PcNa; ▲, BAPC + CdNa. Each point represents the mean of two experiments. The amount of CdNa or PcNa addition was 20% (w/w) to total weight of BAPC and the polymer.

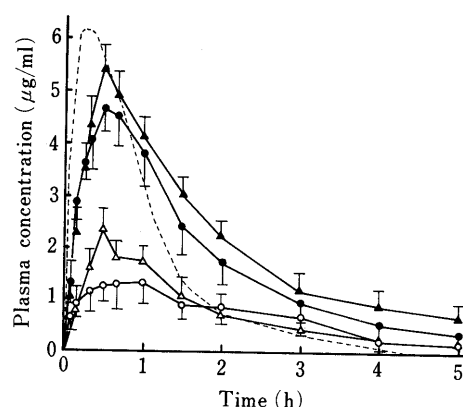


Fig. 5. Plasma Concentration versus Time Curves of Ampicillin after Rectal Administration of Suppositories Formulated with the Physical Mixture and the Adduct in Rabbits

○, BAPC-Pc adduct; △, BAPC-Cd adduct; ●, BAPC + PcNa (20%); ▲, BAPC + CdNa (20%); -----, intact BAPC. Each point represents the mean \pm S.D. of four rabbits. All experiments were carried out at the dose of 17.0 mg/kg of BAPC.

and 17.1 ± 4.1 min), as shown in Tables I and II.

These low C_{max} and late t_{max} values correspond well to the slow permeation and release of BAPC from the adducts and their suppositories as shown in Figs. 2 and 4. These bioavailability parameters are similar in magnitude to the values observed for an adduct of BAPC with Alg ($2.44 \pm 1.20 \mu\text{g/ml}$ and 60.0 ± 0.0 min).¹⁰ Further, these results suggest that the adduct possesses disadvantageous properties (poor wettability and poor solubility in water) for absorption as described above.

Thus, $[AUC]_0^\infty$ became significantly smaller [about 42.4% (BAPC-Pc) or 46.5% (BAPC-Cd) of that obtained from the rectal administration of BAPC alone]. In spite of the low C_{max} and the small $[AUC]_0^\infty$ values, each MRT value was similar to that of BAPC alone, as shown in Table II. This indicates that the sustained release of BAPC from the adduct suppository is reflected in the prolonged rectal absorption of BAPC (Fig. 4a).

Effect of Polymer Addition on Prolonged Rectal Absorption of BAPC The plasma concentrations of ampicillin following the administration of the physical mixture of BAPC and Pc (or Cd) are also shown in Fig. 5. The bioavailability parameters obtained from these plasma drug concentrations are summarized in Table II.

TABLE II. Bioavailability Parameters of Bacampicillin (Dose 17.0 mg/kg) after Rectal Administration of BAPC-Pc, BAPC-Cd Adduct or the Mixture

Composition		C_{\max}^a ($\mu\text{g/ml}$)	t_{\max}^a (min)	MRT^a (h)	$[AUC]_0^\infty^a$ (h \cdot $\mu\text{g/ml}$)	EBA ^b (%)
Adduct	BAPC-Pc	1.29 ± 0.48^c	46.7 ± 13.1^c	1.71 ± 0.05	3.39 ± 0.30^c	18.7
	BAPC-Cd	2.30 ± 0.98^c	36.7 ± 5.8^c	1.85 ± 0.48	3.72 ± 0.19^c	20.6
	BAPC + PcNa (10%)	3.31 ± 1.21^d	37.5 ± 7.1	$1.96 \pm 0.13^{c,d}$	6.84 ± 1.97^d	37.8
	(20%)	4.64 ± 1.00^d	36.5 ± 5.8	$2.05 \pm 0.24^{c,d}$	8.49 ± 1.82^d	46.9
	(40%)	2.27 ± 0.33^d	40.0 ± 6.0	1.38 ± 0.17^d	3.74 ± 0.80	20.7
Mixture	BAPC + CdNa (10%)	4.02 ± 1.99^e	28.0 ± 4.3	1.26 ± 0.33	6.47 ± 1.62^f	35.7
	(20%)	5.39 ± 1.19^f	33.3 ± 5.8	$2.54 \pm 0.48^{c,e}$	$11.96 \pm 1.30^{c,f}$	66.1
	(40%)	3.20 ± 1.30	43.3 ± 5.3	1.44 ± 0.11	5.91 ± 1.57^f	32.1
	BAPC + HPC (20%)	3.59 ± 0.59	30.0 ± 0.1	1.18 ± 0.12^g	3.31 ± 0.76	18.3

a) Each value represents the mean \pm S.D. of four rabbits. C_{\max} and t_{\max} were extracted from a table of peak concentrations. b) Extent of bioavailability: as shown in Table I. Statistically significant differences: c) $p < 0.01$ vs. BAPC in Table I; d) $p < 0.01$ vs. BAPC-Pc adduct; e) $p < 0.05$; f) $p < 0.01$ vs. BAPC-Cd adduct; g) not significant vs. BAPC alone in Table I.

As shown in Fig. 5, absorption rates of BAPC from the suppositories containing the mixture were fast, like that from the suppository containing BAPC alone. However, very large differences in plasma concentration between the rectal administration of BAPC alone and the adducts were observed after t_{\max} ; the plasma concentration of ampicillin due to the adduct administration was maintained higher level than that due to only BAPC administration from about 40 to 300 min. The absorption rate and prolonged absorption were affected by the amount of Pc or Cd addition as indicated in terms of t_{\max} and MRT in Table II. The values increased with increasing polymer addition up to 20%, but the MRT value decreased with 40% addition of the polymer. In these ranges of polymer addition studied, the best bioavailability and the highest C_{\max} were obtained by 20% addition of each polymer, particularly by the addition of Cd. These bioavailabilities are satisfactory, that is, the bioavailabilities due to Pc and Cd addition are 106.1 and 149.5% of that in the case of the administration of BAPC alone. The decrease of the bioavailability by the 40% addition of each polymer seems to be largely due to the inhibitory effect of an increase of the viscosity on the absorption, as discussed below.

The above results, which reveal rapid and prolonged absorption regardless of the similar release behavior of BAPC from suppositories containing the adduct and the mixture (Fig. 4), can be accounted for as follows. As the suppository containing the mixture dissolves in the rectum, BAPC and the polymer are released simultaneously, and the rapid absorption of BAPC begins, followed by the formation of the adduct and by prolonged absorption owing to sustained release of BAPC from the resultant adduct. This view is supported by the result observed in the case of HPC addition (Table II). In contrast with Pc or Cd, no precipitates separated out even when HPC aqueous solution (2%) was added dropwise to 0.04 M BAPC aqueous solution at 0°C. Despite the relatively high C_{\max} level ($3.59 \pm 0.59 \mu\text{g/ml}$) due to the administration of the suppositories containing the mixture of BAPC and HPC, the extent of bioavailability was extremely poor (18.3%) and the prolonged rectal absorption was not obtained as indicated by the MRT value shown in Table II. Therefore, the above results suggest that the formation of the adduct is necessary for prolonged rectal absorption of BAPC and that the viscosity at the absorption sites in the rectum has little effect.

Estimation of Prolonged Absorption In the previous

TABLE III. Pharmacokinetic Parameters of Bacampicillin after Rectal Administration of the Adducts and the Mixture

Composition		k_{aa} (h^{-1})	k_e (h^{-1})
BAPC		1.19 ± 0.15^b	4.64 ± 0.11
Adduct	BAPC-Pc	0.43 ± 0.06	4.63 ± 0.09
	BAPC-Cd	0.66 ± 0.10	4.59 ± 0.11
Mixture	BAPC + PcNa (20%)	0.58 ± 0.15	4.60 ± 0.13
	BAPC + CdNa (20%)	0.43 ± 0.13	4.59 ± 0.07
	BAPC + HPC (20%)	1.07 ± 0.12^b	4.66 ± 0.21
	BAPC + AlgNa (30%) ^a	0.51 ± 0.11	4.64 ± 0.06

a) Taken from ref.2. b) Not significant.

paper,^{6,10)} we reported that BAPC was not detected in the blood after BAPC rectal administration (only ampicillin was present in the blood) and that the pharmacokinetics of ampicillin after BAPC administration (17.0 mg/kg) can be adequately characterized by a one-compartment open model. Thus, the absorption rates for the prolonged rectal absorption in this study were evaluated by the Nelson-Wagner method⁹⁾ and the resultant absorption rate constant can be regarded as an apparent first-order rate constant (k_{aa}) with the release process of the drug from the suppository included (Table III).

As shown in Table III, no differences were observed in the elimination rate constants (k_e). The absorption rate constant for the administration of the adduct suppositories or the mixture suppositories was decreased significantly compared to that for the administration of BAPC alone. On the other hand, the difference in the absorption rate constants between BAPC alone and the mixture with HPC was not significant. From the results, it was found that excellent prolonged absorption without any decrease of bioavailability can be obtained by reducing the apparent absorption rate to about a half of that for the administration of BAPC alone. In these successive studies using rabbits, we assumed that the effective plasma level of ampicillin should be maintained above $0.5 \mu\text{g/ml}$.¹²⁾ The time for which an effective plasma level was maintained was about 2 h by using the suppository containing BAPC alone, whereas it was about 4 h and more than 5 h by using the suppository containing the mixture with PcNa and CdNa, respectively as shown in Fig. 5

In conclusion, the results of this study indicate that suppositories containing a mixture of BAPC and acidic polymer in Witepsol H-15 base provide high bioavailability and prolonged rectal absorption without adduct prepara-

tion, and may be practically useful as a sustained-release rectal preparations. Further, the sustained-release behavior due to the adduct formation suggests that wider application of this interaction with acidic poly electrolytes may be feasible to obtain prolonged rectal absorption of other basic drugs.

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