Gastro-Intestinal Absorption of Ethyl 2-Chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate from Different Dosage Forms in Rats and Dogs

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To obtain information as to a suitable formulation of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)-phenyl]propionate (AL-294), an antihyperlipidemic drug of low water solubility, the bioavailability after its oral administration in various dosage forms was evaluated in rats and dogs. After AL-294 was administered orally, AL-294 acid (2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionic acid), which is a metabolite of AL-294, was detected in the plasma. Therefore, absorbability of AL-294 was evaluated using plasma AL-294 acid levels. AL-294 in an oil solution or in a gelatin capsule showed poor absorption, whereas it's absorption was greatly enhanced in the form of an emulsion. The postprandial administration also showed better absorption. The elimination rate of AL-294 acid from the plasma after oral administration of the emulsion was similar to that after intravenous administration of a sodium salt of AL-294 acid.

Keywords oral absorption; emulsion dosage form; oily drug; postprandial absorption

In order to increase the absorption of a drug with a relatively low water solubility, pharmaceutical techniques such as micronization, application of polymorphism, complex formation, and emulsification have been studied.

For example, it has been reported that the absorption of cyclosporine[1] and griseofulvin[2] were increased by reducing the particle size, by using a ground mixture with microcrystalline cellulose,[3] and by emulsification with oil.[4] The absorption of tolbutamide was increased by the formation of a coprecipitate with polyvinyl pyrrolidone.[5] In these examples, the bioavailability after oral administration was improved by enhancing the *in vitro* dissolution rate of poorly water soluble compounds.

Another example is chloramphenicol palmitate whose potency varies with the difference in crystalline form; the bioavailability of the B-form is significantly greater than that of the A-form.[6] In this case, the intact ester which is poorly absorbed from the gastro-intestinal tract must be hydrolyzed by lipase in the small intestine before any significant absorption can take place, and the B-form is hydrolyzed more easily than the A-form.

In the present study, to obtain information as to a suitable formulation of ethyl 2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionate (AL-294), a new antihyperlipidemic drug with low water solubility, the bioavailability after its oral administration in various dosage forms was evaluated in rats and beagle dogs. Futhermore, the pharmacokinetical properties were studied.

Experimental

Materials AL-294 was synthesized in the Chemistry Laboratories of Takeda Chemical Industries, Ltd., (Osaka).[7] Its chemical structure is shown in Chart 1 (A). AL-294 is a viscous oily liquid at room temperature and has a solubility of 1 μg/ml in buffers ranging from pH 3.5 to 7.5. It is soluble up to 200 mg/ml in methyl alcohol or in chloroform. The partition coefficient of AL-294 in octanol *versus* buffers ranging from pH 1.1 to 7.0 is more than 300.

AL-294 acid (2-chloro-3-[4-(2-methyl-2-phenylpropyloxy)phenyl]propionic acid) which is a metabolite of AL-294, was obtained by hydrolyzing AL-294 and was also supplied by the Chemistry Laboratories. Its chemical structure is shown in Chart 1 (B). AL-294 acid is a very viscous liquid and its solubilities in buffers are *ca.* 700 μg/ml at pH 3.3 and ca. 950 μg/ml at pH 7.5.

AL-294 Na (sodium salt of AL-294 acid, Chart 1 (C)), synthesized in the Chemistry Laboratories, is a pale white powder and is freely soluble in water.

Testrosterone (E. Merck), polysorbate 80 (Tween-80 of Kao-Atlas Co., Ltd.), polyoxyethylene derivative of hydrogenated caster oil (HCO-50, Nikko Chemical Co.), block polymer of polyoxyethylene and polyoxypropylene (Pluronic L-64 and F-68, American Cyanamid Co.), polyethylene glycol 4000 (PEG-4000, Sanyo Kasei Co.), and silicon dioxide (Syloid 266, Fuji-Division Chemical Co.) were obtained commercially. All other chemicals were of reagent grade.

Animal Experiments Male beagle dogs, weighing 8.2 to 11.0 kg, were fasted for 18 h prior to and 8 h after the initiation of absorption experiments. The animals were allowed free access to water throughout the experiment. Drug emulsions were administered to the dogs orally *via* a catheter, while hard capsules containing the drug were swallowed with 50 ml of water. Blood samples, 1 ml each, were collected from the brachial vein periodically.

Male Sprague-Dawley rats, weighing 250 to 300 g, were fasted. Liquid dosage forms of the drugs were administered orally *via* a stomach tube, and an AL-294 Na saline solution was injected intravenously into the femoral vein. Blood samples were collected from the tail vein unless otherwise mentioned.

In rats, after the oral administration of AL-294 emulsion, blood was simultaneously withdrawn from the hepatic portal vein and from the *vena cava* at 15 and 30 min postadministration.

In the experiments of the metabolism of AL-294 to AL-294 acid, the AL-294 emulsion was administered into a ligated jejunum. Blood samples were dripped for 60 min continuously through the polyethylene tubing (PE-50) inserted in the mesentric vein corresponding to the ligated jejumum to the cooled chloroform using a modified method of Bar *et al.*[8] Rats were anesthetized, if necessary, with an intraperitoneal injection of a mixture of sodium pentobarbital and sodium phenobarbital at doses of 100 and 50 mg/kg, respectively.

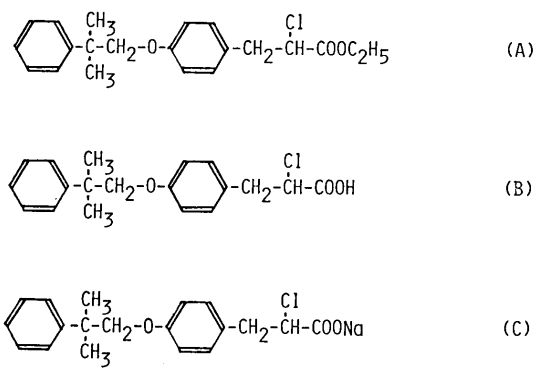

Chart 1. Chemical Structures of AL-294, AL-294 Acid, AL-294 Na

Preparation of Emulsion Emulsions were prepared by an ultrasonic vibration method. The oil phase consisted of 500 mg AL-294 and 500 mg Tween-80, and the aqueous phase was 10 ml of distilled water. After heating both phases to about 50 °C, the aqueous phase was poured into the oil phase under mixing with a magnetic stirrer to make an oil-in-water (o/w) emulsion. After cooling to room temperature, the emulsion was sonicated for 3 min at the vibration rate of 20 kHz with a sonicater (Choh-Onpa Kogyo Co., Ltd.). The mean diameter of the emulsion particles was 2.2 μm, and the particle size distribution was narrow when it was measured with a Coulter Counter model M (Coulter Inc.) using a 30 μm aparture tube. A freeze dry emulsion was prepared by lyophilization of a mixture of mannitol and an emulsion which was prepared by the same procedure as described above.

Determination Procedure Both AL-294 and AL-294 acid were determined by a gas-liquid chromatographic method with a Hitachi Gas-chromatograph K-53 (Hitachi, Ltd.). AL-294 existed as AL-294 acid in plasma samples. AL-294 acid was extracted from plasma and blood samples with chloroform under the acidic conditions of 0.5 N hydrochloric acid solution. After methylating with nitrosomethylurea, it was assayed with a flame ionization detector under the following conditions; the column was 1.0 m long with a 0.38 cm i.d., and packed with 4% OV-17 on Gaschrom Q (80—100 mesh). Operating conditions were; injection temperature, 270 °C; column temperature, 235 °C; flow rate of nitrogen gas, 30 ml/min. Testosterone was used as the internal standard. Under these conditions the retention times (t_R) of AL-294, AL-294 acid methyl ester, and testosterone were 5.2, 4.3, and 7.7 min, respectively. The recovery studies performed with spiked plasma indicated that the extraction was quantitative and the limit of determination was 50 ng. AL-294 and AL-294 acid were separated by partition between chloroform and a 0.5 N sodium hydroxide solution. AL-294 acid in the alkaline solution was reextracted with chloroform under the acidic conditions of 1 N hydrochloric acid solution. The recovery of AL-294 and AL-294 acid was almost 100%.

Results and Discussion

Determination of AL-294 and AL-294 Acid in the Systemic Circulation and in the Portal Vein in Rats AL-294 and AL-294 acid were determined in the blood samples. Ratios of AL-294 and AL-294 acid concentration in plasma and blood are shown in Table I. These results show that about 1% or less of AL-294 absorbed was transferred into the portal vein as unchanged AL-294 while in the systemic circulation all of the drug was present as AL-294 acid.

Also, in dogs, only AL-294 acid was detected in the systemic circulation after the oral administration of AL-294.

Bioavailability of AL-294 after Oral Administration of Various Dosage Forms in Dogs AL-294 in various dosage forms was administered to three dogs. The plasma levels after the oral administration of an emulsion varied among the dogs. However, the plasma levels after an administration of AL-294 in the hard gelatin capsule were very low and the deviation was also small. Therefore, Fig. 1 shows the time courses of the plasma levels of AL-294 acid in the individual dogs after the oral administration of the AL-294 emulsion, and shows the mean time course of the plasma levels of the acid in the three dogs after the oral administration of the drug in a hard gelatin capsule. The peak plasma level (C_{max}) appeared about 3 h postadministration. Area under the plasma concentration–time curve within 8 h ($AUC(0—8)$) of each dosage form and the relative bioavailabilities to the emulsion are shown in Table II. Dosage forms in which a surfactant is used, for example, the emulsion, the mixture with Tween-80 or Pluronic, and freezing dry emulsion, showed comparatively good absorption, but other formulations, such as an adsorbate of AL-294 on Syloid 266 and a mixture of AL-294 and oleic acid, showed low bioavailability.

Effect of the Concentration of Tween-80 on the Absorp-

TABLE I. Ratio of AL-294 and AL-294 Acid Concentration in the Systemic Circulation (*Vena Cava*) and in the Portal Vein after Oral or Intrajejunal Administration in Rats

Time (min)	Ratio of AL-294/AL-294 acid	
	Portal vein	*Vena cava*
15[a]	1.2/98.8	0.4/99.6
15[a]	0.8/99.2	0 /100
30[a]	0.6/99.4	0 /100
30[a]	—	0 /100
0—60[b]	0.9/99.1	—
0—60[b]	0.7/99.3	—

a) Oral administration. b) An administration into the ligated jejunum.

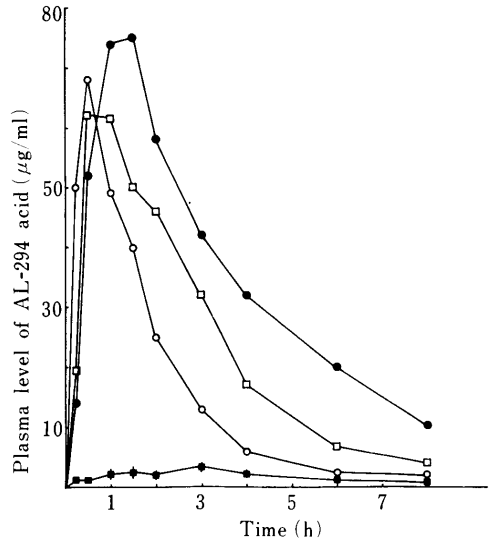

Fig. 1. Plasma Levels of AL-294 Acid in Dogs after Oral Administration of AL-294 in Emulsion or in a Hard Gelatin Capsule at a Dose of 100 mg/dog

●, emulsion form, dog A (IR1M3); □, emulsion form, dog B (GA2M2); ○, emulsion form, dog C (BC4M1); ■, hard gelatin form, (the mean with a standard error of three dogs).

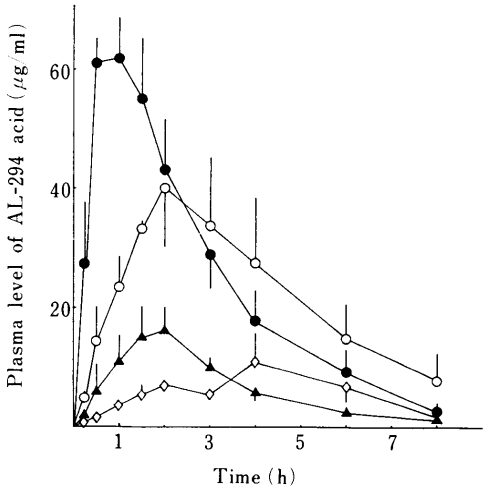

Fig. 2. Plasma Levels of AL-294 Acid in Dogs after Oral Administration of AL-294 as Emulsion or as Physical Mixtures of Various Ratios of Tween-80 and AL-294 in a Capsule at a Dose of 100 mg/dog

●, emulsion; ○, the ratio of Tween-80 to AL-294 (1 : 1); ▲, the ratio of Tween-80 to AL-294 (1 : 0.5); ◇, the ratio of Tween-80 to AL-294 (1 : 0.1). Each value represents the mean with a standard error of three dogs.

TABLE II. *AUC*(0—8) and Relative Bioavailabilities of AL-294 in Various Dosage Forms to Emulsion after Oral Administration of AL-294 at a Dose of 100 mg/Dog

Dosage form	*AUC*(0—8) (μg·h/ml) and relative bioavailability (%) Dog A	Dog B	Dog C	Mean (S.E.)
AL-294 as emulsion	281.4	192.9	109.8	
	(100.0)	(100.0)	(100.0)	(100.0)
AL-294 in capsule	13.1	13.7	—	
	(4.6)	(6.7)	—	(5.7) (1.1)
1 : 1 mixture of AL-294 and oleic acid	81.3	28.4	—	
	(28.8)	(14.7)	—	(21.8) (7.1)
1 : 1 mixture of AL-294 and Tween-80	302.2	154.4	79.4	
	(107.3)	(80.0)	(72.4)	(86.6) (10.6)
1 : 1 mixture of AL-294 and Pluronic L-64	199.4	90.5	93.8	
	(70.8)	(46.9)	(85.4)	(67.7) (11.2)
1 : 1 adsorbed powder of AL-294 on Syloid 266	42.8	31.6	15.8	
	(15.2)	(16.3)	(14.3)	(15.3) (0.6)
1 : 1 : 1 adsorbed powder of AL-294 and Tween-80 on Syloid 266	135.5	55.5	33.6	
	(48.1)	(28.7)	(30.6)	(35.8) (6.2)
1 : 0.2 : 3 mixture of AL-294, Pluronic F-68 and PEG-4000	194.2	53.3	65.0	
	(69.0)	(27.6)	(59.1)	(51.9) (12.5)
1 : 0.5 : 3 mixture of AL-294, Tween-80 and PEG-4000	115.9	92.6	—	
	(41.2)	(48.0)	—	(44.6) (3.4)
Freezing dry emulsion of 1 : 0.1 : 3 mixture of AL-294, Tween-80 and mannitol	169.2	86.2	66.5	
	(60.2)	(44.7)	(60.6)	(55.2) (5.2)

The number in parenthesis represents relative bioavailabilty of each dosage form to emulsion.

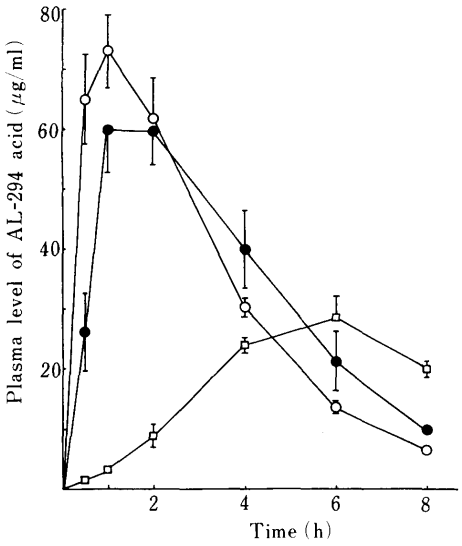

Fig. 3. Plasma Levels of AL-294 Acid in Rats after Oral Administration of AL-294 in Various Dosage Forms at a Dose of 25 mg/kg

○, emulsion; ●, mixture with Tween-80 (2.5 ml/kg); □, oily solution of corn oil. Each point represents the mean with a standard error of five rats.

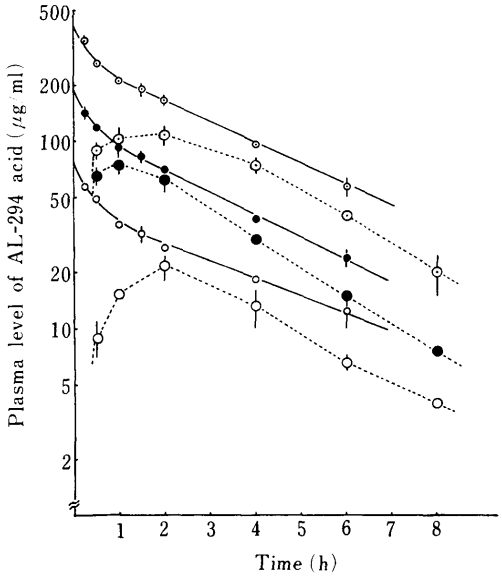

Fig. 4. Plasma Levels of AL-294 Acid in Rats after Intravenous Injection of AL-294 Na or Oral Administration of AL-294 to Rats

Solid lines generated from the simulated equations denote plasma level time curves after intravenous injection, and broken lines denote those after oral administration of AL-294 as emulsion. ⊙, 50 mg/kg; ●, 25 mg/kg; ○, 10 mg/kg. Each value represents the mean with a standard error of five rats.

tion of AL-294 in Dogs Hard gelatin capsules filled with physical mixtures of various ratios of AL-294 and Tween-80 were administered to beagle dogs at a dose of 100 mg of AL-294/dog. Figure 2 shows the plasma level-time curves of AL-294 acid. As shown in Fig. 2, the absorption of AL-294 became more rapid and greater as the concentration of Tween-80 increased. The rate of absorption of AL-294 from the mixture of Tween-80 and the drug (1 : 1) was slower than that from the emulsion form, but the extents of absorption from the two dosage forms were comparable.

Bioavailability of AL-294 after Oral Administration of Various Dosage Forms in Rats Three dosage forms, the emulsion of AL-294, a mixture of AL-294 and Tween-80, and the solution of AL-294 in corn oil, were administered orally to rats. The plasma levels of AL-294 acid are shown in Fig. 3. The plasma level profile of the emulsion indicates that the peak plasma level was attained 2 h later and the level was eliminated 7 h later. The profile was similar to that observed in dogs. The rate and extent of the absorption of AL-294 was largest from the emulsion form. The mixture likely showed slower absorption (*AUC*(0—8)) but the extent was almost the same as that from the emulsion. Since the administration volume of AL-294 alone was too small to administer to the rats, it was diluted with corn oil. The rate and extent of the absorption from the oil solution was the

smallest.

To obtain information on the elimination rate of AL-294 acid from plasma, an aqueous solution of AL-294 Na was intravenously injected into the rats at a dose of 10, 25, or 50 mg/kg. The time courses of the plasma levels are shown in Fig. 4. The data were fitted to the two-compartment open model using linear kinetics. Table III shows the results of the computer analysis. In the dosage range of 10 to 50 mg/kg, the elimination rate constant from plasma, K_{el}, and the distribution volume of the central compartment, V_1, were essentially constant and independent of the dose of AL-294 administered. The correlation between dose and *AUC* calculated was linear as shown in Fig. 5. The plasma levels of AL-294 acid in the rats after the oral administration of various doses of AL-294 in the emulsion form are shown in Fig. 4. The elimination rates from the plasma after the oral administration of an emulsion were similar to those observed following the intravenous injection. The relationship between dose and *AUC* (0—8) after oral administration in the rats was also linear as shown in Fig. 5. These findings indicate that there were no significant non-linear elimination processes, and that the difference in the plasma level between the emulsion and the oil solution was caused by the transfer process from the gestro-intestinal tract to the systemic circulation.

AUC (0—8) after the oral administration of 10, 25, and 50 mg/kg of the AL-294 emulsion were 99.6, 274.4, and 529.2 μg·h/ml, and the absolute bioavailabilities were 57.8, 67.3, and 56.4%, respectively.

Effect of Food on the Absorption of AL-294 AL-294 in a hard gelatin capsule and its emulsion were administered orally to dogs 30 min after a meal, and the plasma levels of AL-294 acid were assayed. As shown in Fig. 6 and Table IV. AL-294 in both dosage forms was absorbed rapidly under postprandial conditions compared with fasting conditions. The extent of absorption from the emulsion was nearly the same under both conditions, whereas the extent of absorption of AL-294 in a hard gelatin capsule in the postprandial was much greater than that in the fasting. The absorption rate of AL-294 from the emulsion most likely increased with a meal. Concerning absorption of AL-294 from the emulsion form in dogs, both the plasma levels of postprandial and fasting crossed each other at 2 h. This

TABLE III. Average Pharmacokinetic Parameters of AL-294 Acid after Intravenous Injection of Various Doses of AL-294 Na to Rats

Parameter	Dose 10 mg/kg	25 mg/kg	50 mg/kg
α (h^{-1})	2.37	3.57	1.50
β (h^{-1})	0.21	0.28	0.23
$0.693/\beta$ (h)	3.3	2.5	3.0
k_{12} (h^{-1})	0.81	1.01	0.38
k_{21} (h^{-1})	1.42	2.43	1.01
k_{el} (h^{-1})	0.35	0.41	0.34
V_1 (l/kg)	0.14	0.13	0.13
AUC(infinity) (μg·h/ml)	208.7	454.2	1138.8
AUC(0—8) (μg·h/ml)	172.4	407.8	973.1

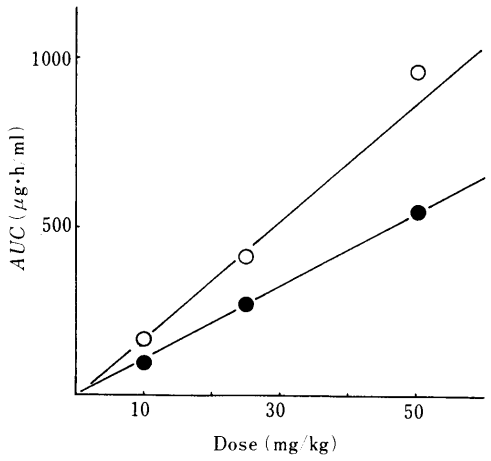

Fig. 5. Relationship between *AUC* and Dose after Intravenous Injection of AL-294 Na or Oral Administration of AL-294 in Rats

○, intravenous injection; ●, oral administration as emulsion.

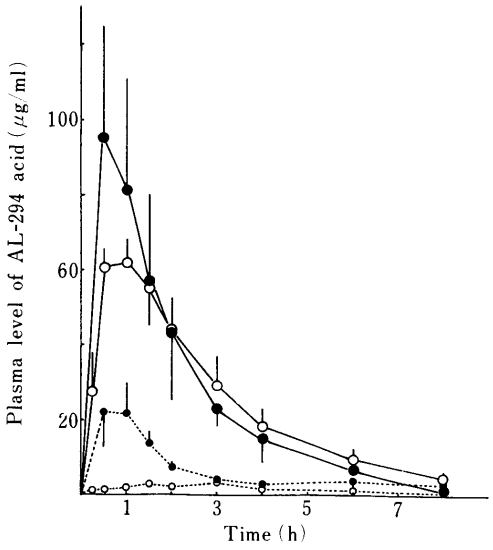

Fig. 6. Plasma Levels of AL-294 Acid in Dogs after Oral Administration of AL-294 in Emulsion or in a Hard Gelatin Capsule at a Dose of 100 mg/dog under Fasting or Postprandial Conditions

Solid lines denote the curves after administration of the emulsion, and broken lines denote those after administration of the hard capsule. ●, postprandial; ○, fasting. Each value represents the mean with a standard error of three dogs.

TABLE IV. *AUC*(0—8) after Oral Administration of AL-294 in Emulsion and in Corn Oil Solution under Fasting and Non-fasting Conditions to Dogs at a Dose of 100 mg/Dog

		AUC(0—8) (μg·h/ml) Dog A	Dog B	Dog C	Mean (S.E.)
Emulsion	Non-fasting	332.4	201.2	97.6	210.4 (67.9)
	Fasting	281.4	193.0	129.3	201.2 (44.1)
Solution	Non-fasting	44.3	59.3	54.8	52.4 (4.7)
	Fasting	14.8	13.7	9.7	12.7 (1.5)

means that AL-294 continued to be absorbed after 2 h in fasting conditions. If AL-294 in the emulsion remained in postprandial in the intestinal lumen, the remaining AL-294 would probably be absorbed and the plasma levels would not cross. But, since plasma levels did actually cross, very little AL-294 remained in the intestinal lumen. It is supposed that most of the AL-294 in the emulsion was absorbed in the dogs. The plasma levels in the postprandial condition seemed to vary more than those in the fasted condition. The cause of the variance is unknown, but we suppose that it was because of differences in bile secretion among the dogs. The extent of enhanced absorption of AL-294 in the hard gelatin capsule and the enhanced absorption rate from the emulsion form with a meal could be attributed to the process of gastro-intestinal absorption, but the exact reasons are not presently clear.

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References

1) B. D. Tarr and S. H. Yalkowsky, *Pharm. Res.*, **6**, 40 (1989).
2) J. R. Marvel, D. A. Schlichting, C. Denton, E. J. Levy, and M. M. Cahn, *J. Invest. Dermatal.*, **42**, 197 (1964).
3) K. Yamamoto, M. Nakano, T. Arita, and Y. Nakai, *J. Pharmacokinet. Biopharm.*, **2**, 487 (1974); T, Arita, *Pharm. Monthly*, **21**, 2737 (1979).
4) S. Kadir, T. Murakami, Y. Higashi, and N. Yata, *Int. J. Pharm.*, **33**, 235 (1986); K. J. Palin, A. J. Phillip, and A. Ning, *ibid.*, **33**, 99 (1986); P. J. Carrigan and T. R. Bates, *J. Pharm. Sci.*, **62**, 1476 (1973).
5) Sekigawa, T. Nagunuma, J. Fujihara, M. Nakano, and T. Arita, *Chem. Pharm. Bull.*, **27**, 31 (1979).
6) A. J. Aguiar, A. W. Kinkel, and J. C. Samyn, *J. Pharm. Sci.*, **56**, 847 (1967).
7) Y. Kawamatsu, H. Asakawa, T. Saraie, K. Mizuno, E. Imamiya, K. Nishikawa, and Y. Hamuro, *Arzneim.-Forsch.*, **30**, 751 (1980); Y. Kawamatsu, H. Asakawa, T. Saraie, E. Imamiya, K. Nishikawa, and Y. Hamuro, *ibid.*, **30**, 585 (1980).
8) W. H. Barr and S. Riegelman, *J. Pharm. Sci.*, **59**, 154 (1970).