[Chem. Pharm. Bull. 33(12)5495—5502(1985)]

Movement of Granules and Tablets in the Gastrointestinal Tract of Gastric-Emptying-Controlled Rabbits

TERUTAKA TAKAHASHI,*,[a] YOSHIMI SHIRAI,[b] YASUHIKO NAKAMURA,[b] YUKO UEZONO,[a] HIROKAZU MAKITA,[b] YUTAKA NAKANISHI,[a] and YU IMASATO[b]

Pharmaceutical Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564, Japan[a] and Ebie 1–5–51, Fukushima-ku, Osaka 553, Japan[b]

(Received April 19, 1985)

The suitability of gastric-emptying-controlled rabbits (GEC-rabbits) as a model for estimating the bioavailability of controlled-release preparations in humans was investigated. Non-disintegrating tablets (diameter of 4.1—7.7 mm) and granules (diameter of 0.8—2.0 mm; specific gravity of 0.90—1.85) were used as a model preparation. Six tablets and 100 granules were administered to GEC-rabbits, and the number of each dosage form remaining in the gastrointestinal tract was counted at suitable intervals.

Tablets with diameters of 4.1 and 5.8 mm were randomly emptied from the stomach and the inter-animal variation was very large. Tablets with a diameter of 7.7 mm were not emptied at all even after 7 h. Granules were gradually emptied from the stomach and the profile was similar to that in humans under non-fasting conditions. The gastric emptying was strongly influenced by specific gravity, but the small intestinal transit time was not influenced by either diameter or specific gravity. The mean transit time through the small intestine (about 1.2 h) was about one-third that of humans.

The results obtained in this study show that it is important to consider duration and extent of absorption in attempting to predict the bioavailability of controlled-release preparations in humans by using GEC-rabbits.

Keywords——tablets; granules; diameter; specific gravity; gastric-emptying-controlled rabbit; gastric emptying; intestinal transit time

Introduction

The bioavailability of a drug from an oral dosage form will be influenced by various pharmaceutical and physiological factors. Controlled-release preparations such as enteric-coated, sustained-release preparation are designed so that a drug will be released from a preparation at a limited segment or through the whole region of the gastrointestinal tract. Accordingly, gastric residence time and intestinal transit time are very important physiological factors affecting the bioavailability of these preparations.[1–4] In the development of controlled-release preparations, the use of humans in the bioavailability studies would provide the most appropriate results. However, the use of humans is impractical in new drug development and preformulation studies because of ethical, economical, analytical and statistical considerations. Therefore, the development of adequate animal models for estimating the bioavailability of oral dosage forms in humans is necessary. Beagle dogs have been used as a model animal in some studies.[5–7] Aoyagi *et al.*[8,9] found that preparations were rapidly emptied from the stomach in dogs as compared with humans and the correlation between humans and dogs as regards the bioavailability of griseofulvin products was poor. Though rabbits can be orally given solid preparations such as tablets and capsules, they have

been considered inadequate as a model animal for estimating drug bioavailability in humans because of the difficulty in obtaining an empty stomach and long-term residence of preparations in the stomach.[10] Recently, rabbits in which gastric emptying was controlled have been developed by Maeda *et al.*[11,12] and the usefulness of rabbits has been demonstrated in bioavailability studies.[11-18] Further, we reported that rabbits in which gastric acidity was controlled, as well as gastric emptying, were useful for bioavailability studies of preparations whose dissolution and stability were influenced by the pH of the gastric contents.[19,20] There have been a few reports[14,18,20] concerning the bioavailability of controlled-release preparations in rabbits.

The present study was undertaken to investigate the transit of non-disintegrating granules and tablets through the gastrointestinal tracts of gastric-emptying-controlled rabbits (GEC-rabbits). The usefulness of rabbits as a model animal for estimating the bioavailability of controlled-release preparations in humans is discussed.

Experimental

Materials——Brilliant blue FCF aluminum lake (Blue 1# Al. lake) and erythrosine aluminum lake (Red 3# Al. lake) were purchased from San-Ei Chemical Industries, Ltd., and glass beads (MK-4GX) and blue spray lacquer were purchased from Shinmaru Enterprises Co., and Rock Paint Co., respectively. Other materials were commercially available.

Preparation of Tablets——Tablet cores were made according to the formula in Table I, using a single compacting machine (Kikusui Seisakusho, 2B). Tablet cores were coated with dichloromethane solution containing 3.8% (w/w) ethylcellulose and 0.2% (w/w) polyethylene glycol 6000 (spray solution) by means of a spraying method in a pan, in order to avoid disintegration. The diameter and specific gravity of tablets are shown in Table I.

Preparation of Granules——Granules with Specific Gravity of 1.85: Glass beads (0.7—1.0 mm in diameter) were precoated with blue lacquer, and coated with spray solution by the same method as used for the tablets. The diameter of granules was in the range of 1.41—1.00 mm, and a mean diameter of 1.2 mm was adopted in this report.

Granules with Specific Gravity of 1.25: Granule cores were made by a wet granulation method according to the formula in Table I, using a high speed mixer (Mitsuimiike Seisakusho, HENSHEL 20B). The granule cores were coated by the same method as used for the tablet. The diameter of granules was in the range of 2.38—1.68 mm (mean, 2.0 mm), 1.41—1.00 mm (mean, 1.2 mm) and 0.84—0.71 mm (mean, 0.8 mm) in different preparations.

Granules with Specific Gravity of 1.01: Ethylcellulose powder (0.05—0.3 mm in particle size) and Red 3# Al. lake were mixed using the high speed mixer and were sprayed with ethanol to form suitable granules. The diameter of the granules was in the range of 1.41—1.00 mm (mean, 1.2 mm).

Granules with Specific Gravity of 0.9: Granules with a specific gravity of 1.01 and whose diameter was in the range of 1.19—0.84 were sprayed with 4% (w/w) ethylcellulose solution in dichloromethane in a pan. The diameter of granules was in the range of 1.41—1.01 mm (mean, 1.2 mm).

Measurement of Swelling of Preparations——The swelling percentage of preparations was calculated from the volume change of preparations after a disintegration test in water for 7 h using the JP X equipment. All preparations showed virtually no swelling (Table I) or deformation.

Specific Gravity of Preparations——The specific gravity of preparations was calculated from the volume measured in water at 25°C and the weight of preparations.

GEC-Rabbits——Gastric emptying of rabbits was controlled by a slight modification of the previous method.[19] White male rabbits, weighing about 3 kg, were used for this experiment. After fasting overnight, the rabbits were lightly anesthetized by intravenous injection of pentobarbital sodium (15 mg/kg), and the stomachs were washed with about 200 ml of warm saline (37°C) using a rubber stomach tube. After the gastric lavage, the rabbits were fed 100 g of CR-S (Nihon Clea Co.) per day for 5 d. After fasting overnight again, the rabbits were fed 100 g of soft CR-S (40 g of CR-S: 60 g of water) per day for 2 d. On the day of the experiment, the rabbits were fed 50 g of soft CR-S. During gastric emptying control and the administration studies, the rabbits were allowed water freely, and a cangue was fixed to the neck to prevent coprophagy.

Administration Studies——On the day of the experiment, rabbits were fed 50 g of soft CR-S at 9:00—10:00 a.m. At 5—10 min after the end of feeding, a test preparation was administered to the rabbits. Six tablets were put on the radix linguae, which was pulled out using tweezers, and were swallowed, and immediately 100 granules were passed into the stomach with 20 ml of water through a rubber tube. The administration of preparations was divided into three groups: the first group received tablets with diameters of 4.1 and 5.8 mm, and three kinds of granules with a specific gravity of 1.25 (diameters of 0.8, 1.2 and 2.0 mm); the second group received four kinds of granules with a diameter of 1.2 mm (specific gravities of 0.90, 1.01, 1.25 and 1.85); the third group received only tablets with a

TABLE I. Characteristics and Formulae of Tablets and Granules

	Specific gravity	Diameter (mm)	Thickness (mm)	Swelling %[a]	Formula		
					Core		Coating layer
Tablets	1.22	4.1	2.0	5>	Lactose HMS	(69.35)	
					Corn starch	(18.00)	
	1.23	5.8	3.1	5>	Avicel 101	(10.00)	Ethocell (95)
					Blue #1 Al lake	(0.15)	PEG 6000(5)
	1.29	7.7	5.3	5>	HPC L	(2.00)	
					Mg stearate	(0.50)	
Granules	1.85	1.2		5>	Glass beads		
					Blue lacquer		
	1.25	2.0		5>	Corn starch	(75.9)	
		1.2		5>	Avicel 101	(20.0)	Ethocell (95)
					Blue #1 Al lake	(0.1)	PEG 6000(5)
		0.8		5>	TC-5 R	(4.0)	
	1.01	1.2		5>	Ethocell 100	(99.8)	
	0.90	1.2		5>	Red #3 Al lake	(0.2)	Ethocell (95)
							PEG 6000(5)

a) % volume increase after disintegration test for 7 h.

diameter of 7.6 mm. At 1.5, 3, 5 and 7 h after administration, rabbits were sacrificed, the stomach and the small intestine were immediately isolated, and the number of preparations remaining in the gastrointestinal tract was counted. The small intestine was partitioned into three regions: the upper (80 cm), the middle (70—110 cm), and the lower small intestine (80 cm). The number of preparations in the region below the cecum was calculated by subtraction of the number remaining in the stomach and the small intestine from the number administered.

Mean Transit Time[21]——The transit of granules in the gastrointestinal tract was expressed in terms of the time required for 50% to leave the stomach (mean gastric emptying time) and for 50% to arrive at the cecum (mean cecal arrival time). The mean gastric emptying time was obtained by interpolating or extrapolating the linear portion of log (remaining %) plots against time, and the mean cecal arrival time was determined graphically from the curves of log (arrival %) plots against time. The difference between these two values was taken as representing transit in the small intestine (mean small intestinal transit time).

Results and Discussion

Gastric Emptying of Tablets

Figure 1 shows the number of tablets remaining in the stomach at various times after oral administration of six tablets with different diameters (4.1, 5.8 and 7.6 mm) to GEC-rabbits. The smallest (4.1 mm) tablets were not emptied from the stomach at 1.5 h after administration but were partly emptied after 3 h. The gastric emptying varied considerably between animals and was irregular. Tablets with a diameter of 5.8 mm were partly emptied after 5 h. As with the smallest tablets, the gastric emptying varied considerably between animals and was irregular. Tablets with a diameter of 7.7 mm were not emptied at all even after 7 h.

Thus, it was found that tablets were emptied irregularly from the stomach of GEC-rabbits with large inter-animal variation. Further, the limiting size of tablets which could be emptied was less than 7.7 mm in diameter. It was reported that gastric emptying of tablets varied considerably within and between subjects with an "all or none" effect when single-unit preparations such as enteric-coated tablets which are not disintegrated in the stomach were given to humans.[22-27] Similar results were obtained in GEC-rabbits. However, there are many commercially available tablets whose diameters are greater than the maximum that can be emptied in GEC-rabbits. Therefore, the bioavailability must be carefully estimated, taking into account inter-animal variation and the diameter of tablets, when the bioavailability of

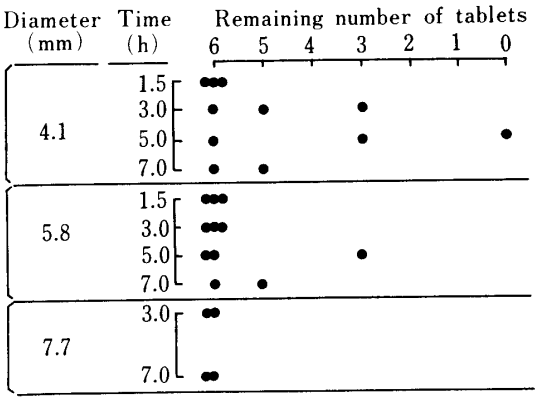

Fig. 1. Number of Tablets Remaining in the Stomach after Oral Administration of Tablets with Different Diameters to GEC-Rabbits

Each point represents the results in an individual animal.

TABLE II. Distribution Number of Tablets in the Gastrointestinal Tract

Diameter (mm)	Time (h)	Animal	Distribution number of tablets in GI[a]				
			Stomach	Small intestine			Below cecum
				Upper	Middle	Lower	
4.1	3.0	1	6				
		2	5	1			
		3	3		2		1
	5.0	1	6				
		2	3				3
		3	0				6
	7.0	1	6				
		2	5		1		
5.8	5.0	1	6				
		2	6				
		3	3				3
	7.0	1	6				
		2	5		1		

a) GI: gastrointestinal tract.

enteric-coated tablets or sustained release tablets whose absorption rate is limited by the gastric emptying rate is investigated using GEC-rabbits.

Distribution of Tablets in the Gastrointestinal Tract

Table II shows the distribution of tablets in the gastrointestinal tract in the cases where tablets were emptied from the stomach. The distribution of tablets in the small intestine was erratic (Table II). From the results on gastric emptying, it appears that irregular gastric emptying of tablets is responsible for this erratic distribution. It has been reported that the gastrointestinal transit time from the mouth to the cecum of single-unit preparations varied markedly in the range from 1 to 16 h,[28] or 5 to 40 h.[29] However, we can not discuss the small intestinal transit time of tablets used in this study, because the tablets were not traced individually.

Influence of Diameter on Gastrointestinal Transit of Granules

Figure 2 shows the gastrointestinal transit of three kinds of granules (diameters of 0.8, 1.2 and 2.0 mm, respectively) with a specific gravity of 1.25. Each type of granules was gradually emptied from the stomach. The emptying rate, though not significantly different, tended to increase with decreasing diameter of granules. The inter-aimal variation was quite small as compared with that in the case of tablets. In general, it is well known that inter-subject

variation in gastric emptying of sub-unit or multiple-unit preparations is small as compared with that for single-unit preparations. It was reported that multiple-unit preparations were gradually emptied from the stomach in humans with a mean gastric emptying time of about 1.5 h under fasting[30] or about 6 h under non-fasting conditions.[31] Gastric emptying profiles of granules obtained in this study were smilar to those of granules in humans who had received usual meals.[2] Further, the mean gastric emptying time (3.7—5.0 h) (Table III) agreed very well with the results (mean ±S.E., 4.75±0.65 h) in humans[32] who had received pellets having a diameter of 0.7—1.2 mm and specific gravity of 1.25 with a heavy breakfast. Accordingly, these results indicate that it is possible to use GEC-rabbits for estimating the bioavailability of controlled-release granules such as enteric-coated and sustained-release granules in humans under certain conditions, namely when food intake is permitted.

Granules of each kind began to arrive at the cecum within 1.5 h after administration, though the amount was small. Thus, the transit of granules through the small intestine is very fast. The distribution of granules in the small intestine was not particularly influenced by the diameter. The gastrointestinal transit time from the mouth to the cecum was not significantly different between granules. Similarly, Bechgaard and Ladefoged[33] reported that the gastrointestinal transit time of the pellets in humans was not influenced by diameter (0.3—0.7 and 1.2—1.7 mm).

The amount of granules staying in the small intestine was always 5—10% of granules administered. It appears, therefore, that granules, irrespective of their diameter, transit through the small intestine at an approximately constant speed. The mean small intestinal transit time of granules, which is taken as the difference between mean cecal arrival time and mean gastric emptying time, was in the range of 1.0—1.2 h (Table III), while the transit time of pellets with a specific gravity of 1.2 and a diameter of 0.7—1.2 mm in humans, reported by Davis *et al.*,[21,32] was in the range of 3—4 h. The anatomical length of the small intestine in the rabbits used in this study was about 2.5 m, being about two-fifths of that of humans (6—7 m). If the small intestine of rabbits were of the same length as that of humans, the mean transit

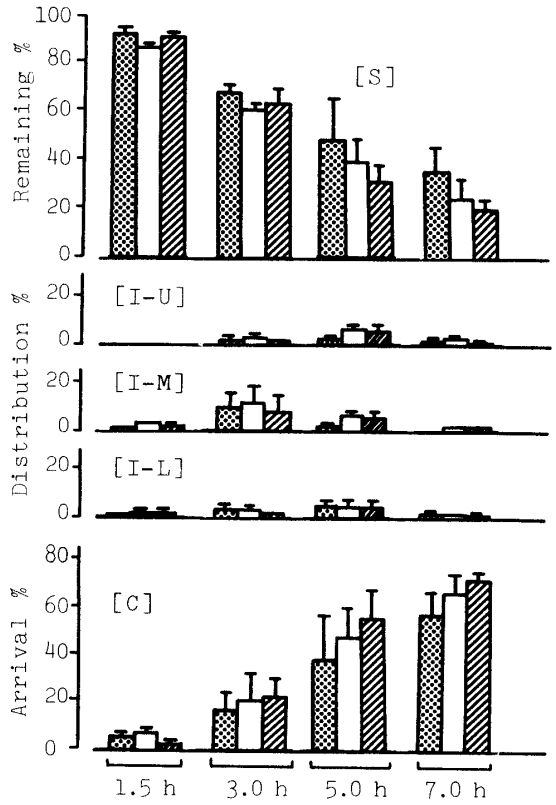

Fig. 2. Gastrointestinal Transit of Granules with Different Diameters in GEC-Rabbits

Each column represents the mean±S.E. ($n=3$ at 1.5, 3.0 and 5.0 h; $n=4$ at 7.0 h). [S], stomach; [I-U, M, L], upper, middle and lower region of the small intestine; [C], cecum. Diameter: ▩, 2.0 mm; □, 1.2 mm; ▨, 0.8 mm.

time of granules in GEC-rabbits would be about 3 h, similar to that of humans. However, these results indicate that it is important to consider duration and extent of absorption in estimating the bioavailability of time-dependent release preparations in humans using GEC-rabbits, since the absolute transit time of granules in GEC-rabbits is about one-third of that of humans.

Influence of Specific Gravity on Gastrointestinal Transit of Granules

Figure 3 shows the gastrointestinal transit of four kinds of granules (specific gravities of 0.9, 1.01, 1.25 and 1.85) with a diameter of 1.2 mm. Granules with a specific gravity of 1.01 were rapidly emptied from the stomach as compared with the other three granules. The mean gastric emptying time was 2.3 h (Table III) and is similar to that of gastric contents[19] (about 1.9 h). It appears, therefore, that the granules are emptied together with the gastric contents. On the other hand, granules with a specific gravity of more or less than 1.01 were emptied slowly as compared with granules having a specific gravity of 1.01. These results indicate that gastric emptying of granules in GEC-rabbits, as well as humans,[34] is influenced by specific gravity.

Rabbits were sacrificed 3 h after administration of granules and the stomach was cut along the small curvature. Granules with a specific gravity of 0.9 were floating in the liquid phase of the gastric contents, granules with a specific gravity of 1.01 or 1.25 were mixed with gastric contents, and granules with a specific gravity of 1.85 were found in the lower layer of the gastric contents. These observations suggest that gastric emptying of granules with a specific gravity of 0.9 is delayed because of late arrival at the pylorus owing to the floating behavior. On the other hand, gastric emptying of granules with a specific gravity of 1.85 may be delayed because movement of the sinking granules to the pylorus was hindered by gastric contents.

The distribution of granules in the small intestine was not particularly influenced by the

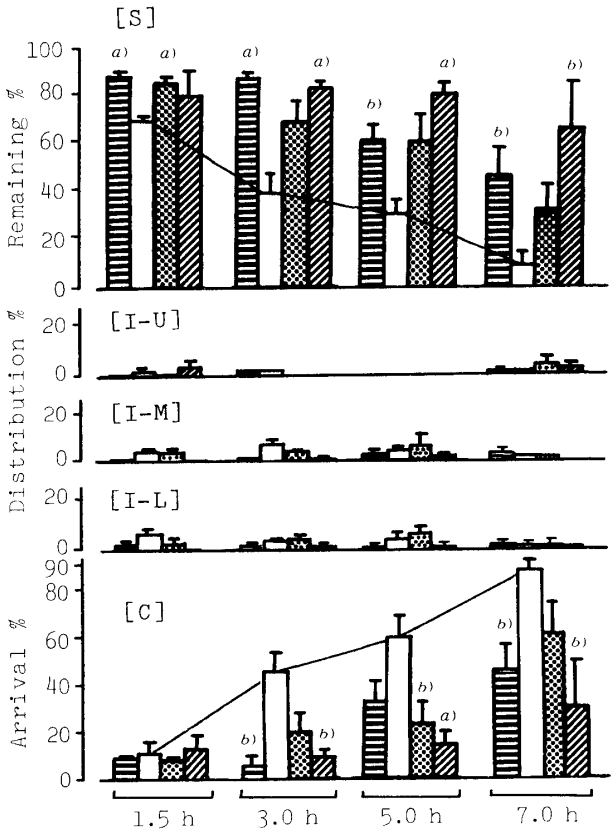

Fig. 3. Gastrointestinal Transit of Granules with Different Specific Gravities in GEC-Rabbits

Each column represents the mean ± S.E. ($n=3$ at 1.5 and 3.0 h; $n=4$ at 5.0 and 7.0 h). [S], stomach; [I-U, M, L], upper, middle and lower region of small intestine; [C], cecum. Specific gravity: ▨, 1.85; ▩, 1.25; □, 1.01; ▤, 0.90. *a*) $p<0.01$ against □. *b*) $p<0.05$ against □.

TABLE III. Gastrointestinal Transit of Granules

Specific gravity	Diameter (mm)	Mean transit time (h)		
		Gastric emptying	Cecal arrival	Small intestinal transit
0.90	1.2	5.9	7.5[a]	1.6
1.01	1.2	2.3	3.6	1.3
1.25	0.8	3.7	4.8	1.1
	1.2	4.8	5.8	1.0
	2.0	5.0	6.2	1.2
1.85	1.2	8.0[a]	9.3[a]	1.3

a) Extrapolated value since less than 50% had been emptied from the stomach and had arrived at the cecum within 7 h.

specific gravity at any time. The amount of granules left in the small intestine was small and remained approximately constant. This suggests that granules, irrespective of their specific gravity, transit through the small intestine at an approximately constant speed. As shown in Table III, the mean small intestinal transit times of granules with specific gravities of 0.90, 1.01, 1.25 and 1.85 were 1.6, 1.3, 1.0 and 1.3 h, respectively. These results indicate that the small intestinal transit time of granules is not particularly influenced by specific gravity or diameter, though the gastric emptying time of granules was strongly influenced by specific gravity.

Bechgaard and Ladefoged[33] have reported that the gastrointestinal transit time (from the mouth to the ileo-cecal junction) of pellets increased with increase of the spcific gravity from 1.0 to 1.6, irrespective of diameter, when humans received four kinds of pellets with different specific gravities (1.0 and 1.6) and diameters (0.3—0.7 and 1.2—1.7 mm). However, it is not evident in the case of humans whether the increase of gastrointestinal transit time of pellets with the increase of specific gravity depends mainly on gastric emptying or small intestinal transit, since the movement of pellets in the stomach and the small intestine has not been observed separately. In the case of GEC-rabbits, gastric emptying was predominantly responsible for the influence of specific gravity on gastrointestinal transit of granules. It is well known that gastric emptying of oral dosage forms is greatly influenced by physiological factors, particularly food intake, in addition to pharmaceutical factors such as size, shape and specific gravity of preparations.[35] However, it has been reported that the mean transit time of different preparations through the small intestinal tract in humans was 3—4 h, irrespective of dosage form (tablet–pellets)[21] or quantity of food (light–heavy breakfast).[32] Thus, it seems likely that the small intestinal transit time of oral dosage forms is not influenced by specific gravity. However, further investigations are required to confirm this.

Results obtained in this study indicate that, provided differences between humans and rabbits in the duration and extent of absorption (related to the difference of the anatomical length of the small intestine) are taken into consideration, it is possible to use GEC-rabbits for predicting the bioavailability of controlled-release preparations in humans.

Acknowledgement We are grateful to Drs. M. Shimizu and M. Kono, Dainippon Pharmaceutical Co., Ltd. for advice and helpful discussions.

References

1) H. Maekawa, Y. Takagishi, Y. Doi, and K. Iwamoto, *Yakuzaigaku*, **30**, 94 (1970).

2) H. Maekawa, Y. Takagishi, Y. Doi, and K. Iwamoto, *Yakuzaigaku*, **30**, 102 (1970).
3) M. J. Dew, P. J. Hughes, M. G. Lee, B. K. Evans, and J. Phodes, *Br. J. Clin. Pharm.*, **14**, 405 (1982).
4) C. Bogentoft, M. Alpsten, and G. Ekenved, *J. Pharm. Pharmacol.*, **36**, 350 (1983).
5) H. L. Newmark, J. Berger, and J. T. Carstensen, *J. Pharm. Sci.*, **59**, 1249 (1970).
6) W. A. Cressman and D. Sumner, *J. Pharm. Sci.*, **60**, 132 (1971).
7) J. W. Poole, *Rev. Can. Biol.*, (Suppl.), **32**, 43 (1973).
8) N. Aoyagi, H. Ogata, N. Kaniwa, M. Koibuchi, T. Shibazaki, A. Ejima, N. Tamaki, H. Kamimura, Y. Katougi, and Y. Omi, *J. Phrm. Sci.*, **71**, 1169 (1982).
9) N. Aoyagi, H. Ogata, N. Kaniwa, and A. Ejima, *J. Pharmacobio-Dyn.*, **7**, S-74 (1984).
10) W. L. Chiou, S. Riegelman, and J. R. Amberg, *Chem. Pharm. Bull.*, **17**, 2170 (1960).
11) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **66**, 69 (1977).
12) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *Chem. Pharm. Bull.*, **27**, 3066 (1979).
13) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *J. Pharm. Sci.*, **68**, 1286 (1979).
14) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *Yakuzaigaku*, **40**, 13 (1980).
15) E. Gu and M. Matsumoto, *Yakuzaigaku*, **40**, 94 (1980).
16) T. Maeda, H. Takenaka, Y. Yamahira, and T. Noguchi, *Chem. Pharm. Bull.*, **28**, 2824 (1980).
17) N. Aoyagi, H. Ogata, N. Kaniwa, and A. Ejima, *J. Pharmacobio-Dyn.*, **7**, 630 (1984).
18) M. Komatsu, K. Tagawa, M. Kawata, and S. Goto, *Chem. Pharm. Bull.*, **31**, 262 (1983).
19) T. Takahashi, Y. Uezono, and H. Fujioka, *Yakuzaigaku*, **43**, 61 (1983).
20) T. Takahashi, M. Mori, Y. Uezono, H. Fujioka, and Y. Imasato, *Yakuzaigaku*, **43**, 187 (1983).
21) S. S. Davis, J. G. Hardy, M. J. Taylor, D. R. Whalley, and C. G. Wilson, *Int. J. Pharmaceut.*, **21**, 167 (1984).
22) R. H. Blythe, G. M. Grass, and D. R. MacDonnell, *Am. J. Pharm.*, **131**, 206 (1959).
23) J. G. Wagner, W. Veldkamp, and S. Long, *J. Am. Pharm. Assoc.*, **49**, 128 (1960).
24) J. R. Leonards and G. Levy, *J. Am. Med. Assoc.*, **193**, 99 (1965).
25) B. Hulme, V. H. T. James, and R. Rault, *Br. J. Clin. Pharmacol.*, **2**, 317 (1975).
26) C. Bogentoft, I. Carlsson, G. Ekenved, and A. Magnusson, *Eur. J. Clin. Pharmacol.*, **14**, 351 (1978).
27) R. G. Henderson, T. Wheatley, J. English, J. Chakraborty, and V. Marks, *Br. Med. J.*, **1**, 1534 (1979).
28) R. P. Rosswick, R. D. Stedeford, and B. N. Brooke, *Gut*, **8**, 195 (1967).
29) H. Bechgaard and K. Ladefoged, *J. Pharm. Pharmacol.*, **33**, 791 (1981).
30) C. Bogentoft, C. Appelgren, U. E. Jonsson, and J. Sjogren, Abstract of Papers, Annual Meeting of the Swedish Pharmaceutical Society, 1981, p. 109.
31) R. E. Horton, F. G. M. Ross, and G. H. Darling, *Br. Med. J.*, **1**, 1537 (1965).
32) S. S. Davis, J. G. Hardy, M. J. Taylor, D. R. Whalley, and C. G. Wilson, *Int. J. Pharmaceut.*, **21**, 331 (1984).
33) H. Bechgaard and K. Ladefoged, *J. Pharm. Pharmacol.*, **30**, 690 (1978).
34) H. Maekawa, Y. Takenaka, Y. Doi, and T. Ogura, Abstract of Papers, The 96th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, 1976, p. 171.
35) H. Bechgaard and F. N. Christensen, *Pharm. J.*, **229**, 373 (1982).